

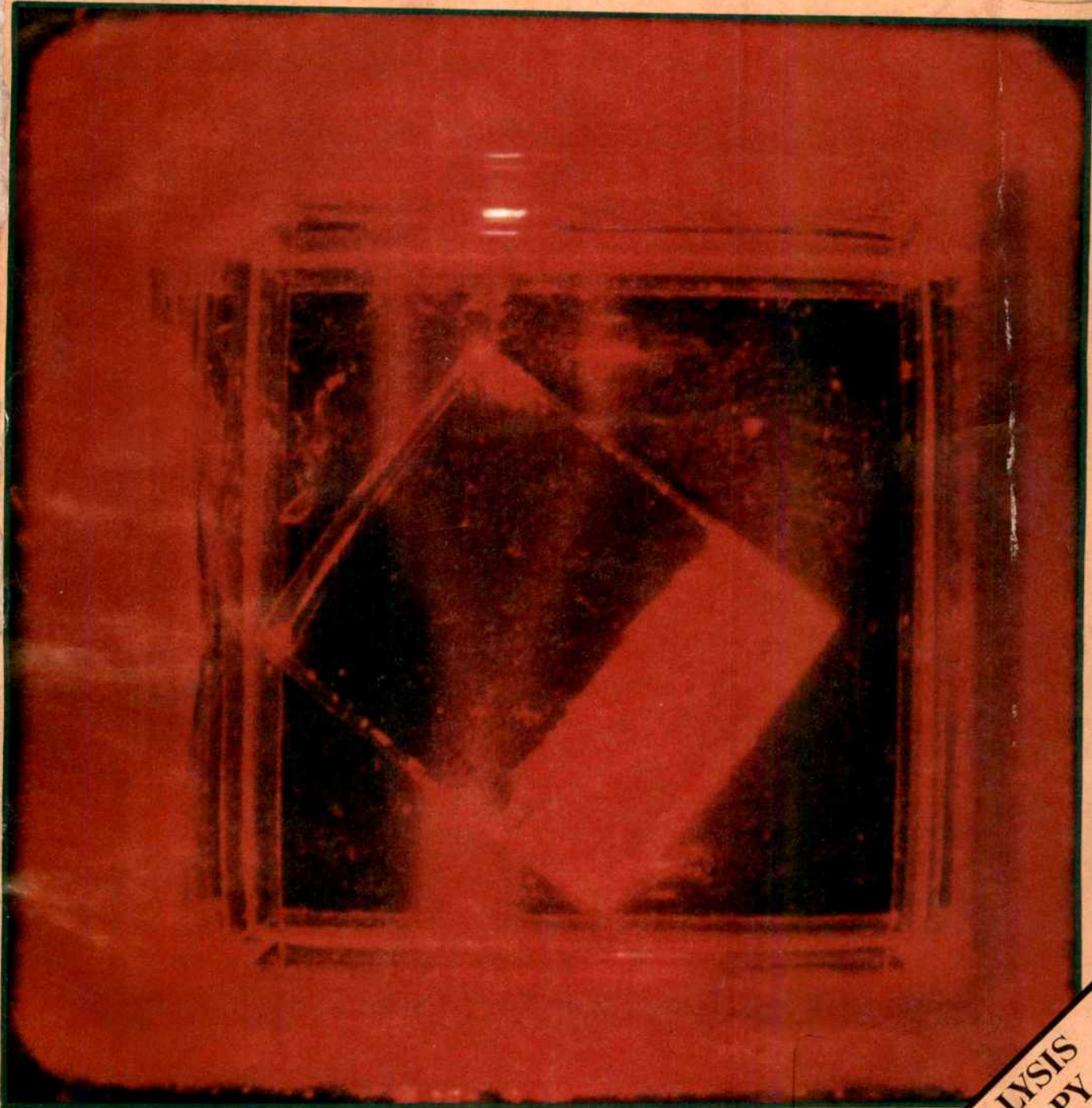
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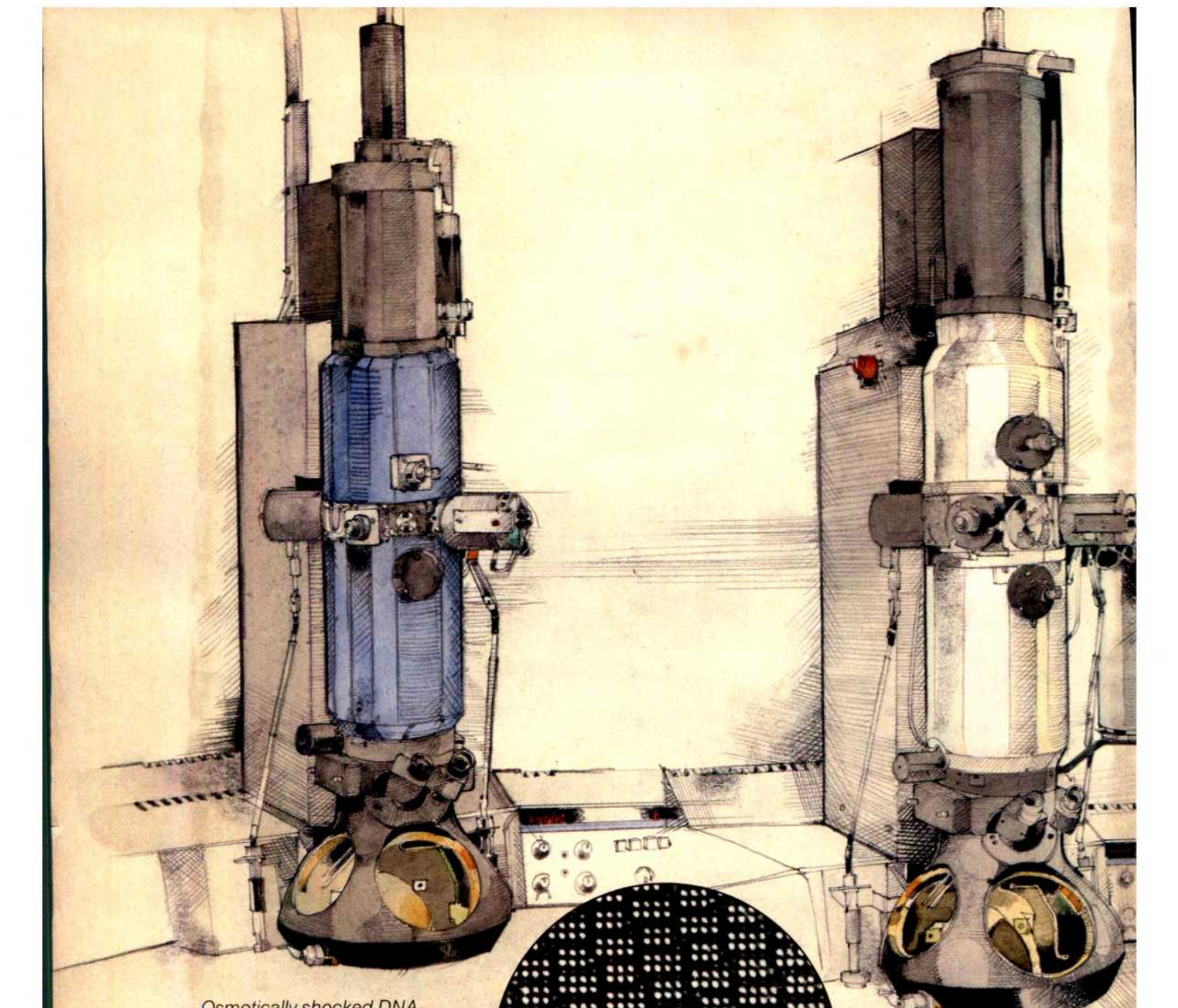
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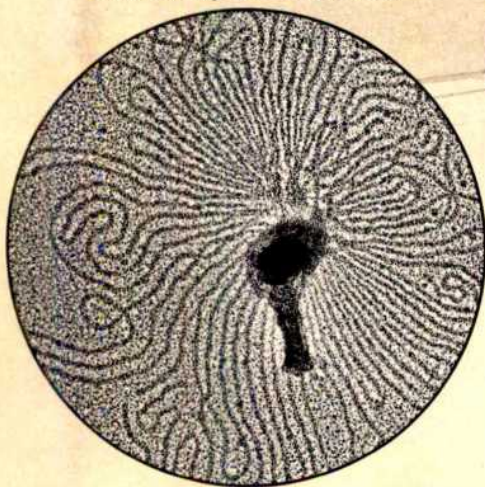


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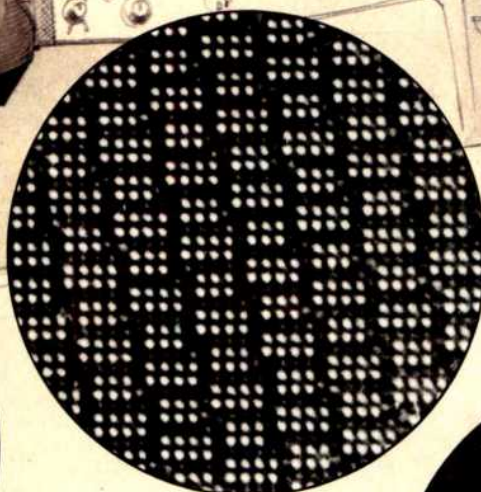
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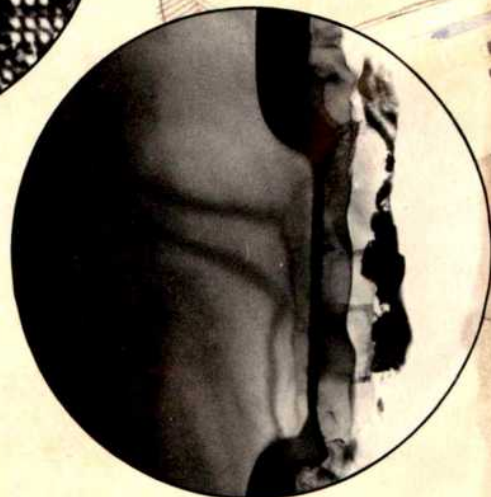
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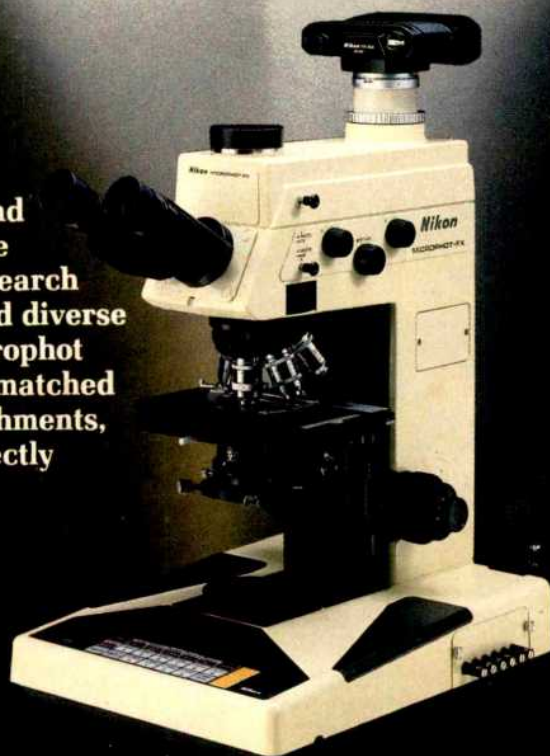
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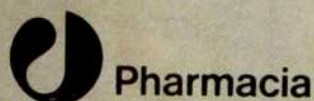
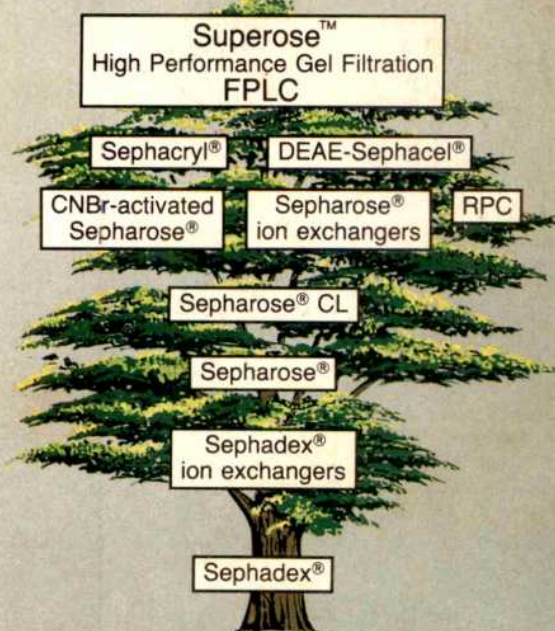
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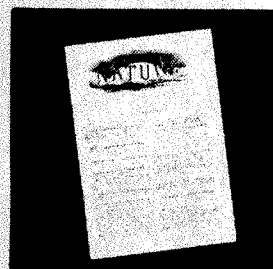
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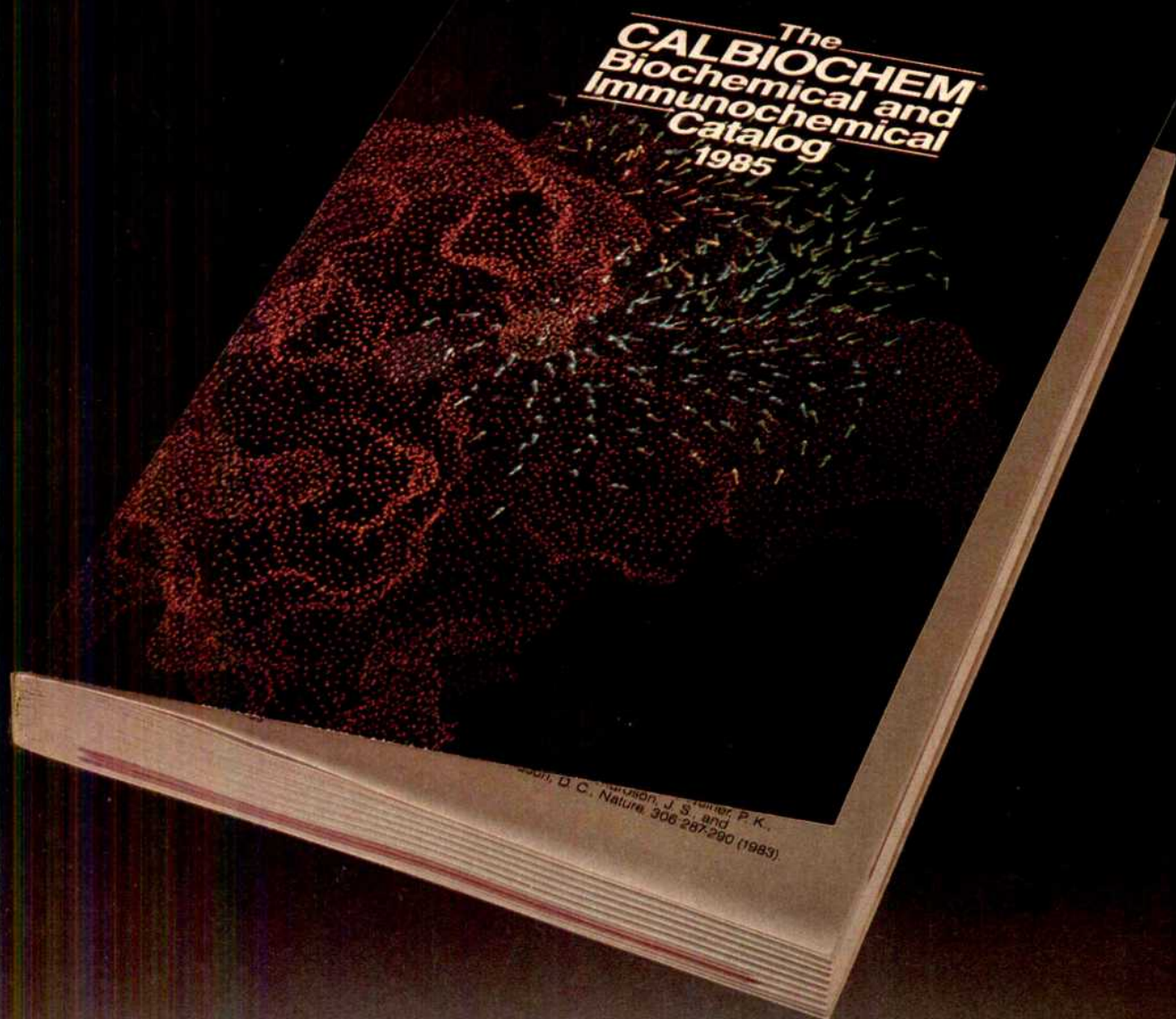
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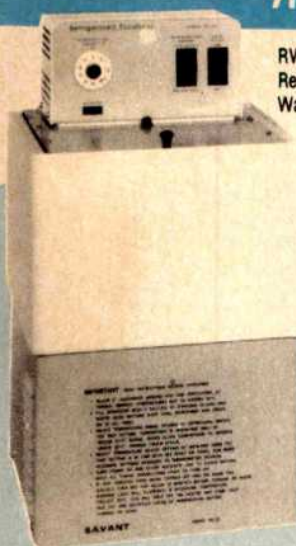
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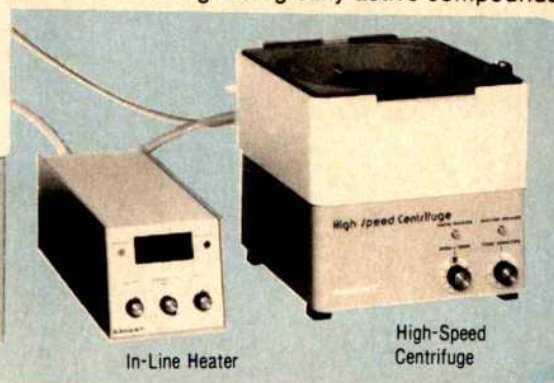
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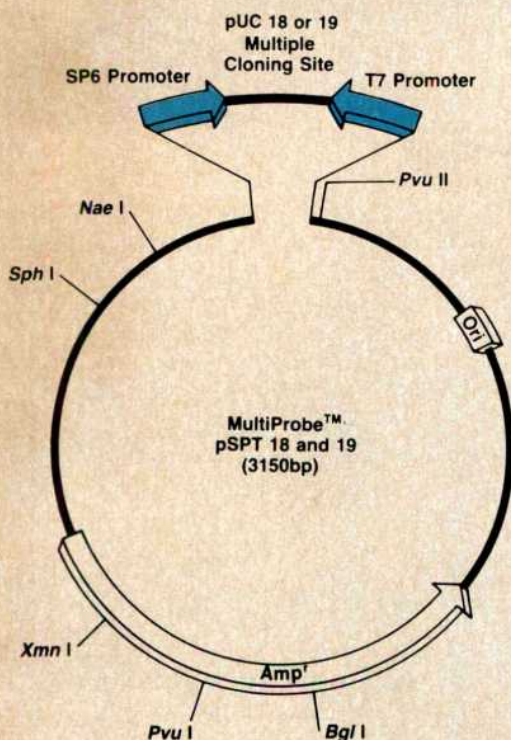
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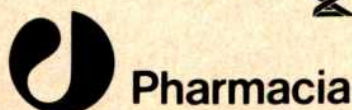


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European setback at Milan

Disagreement among members of the European Communities last weekend at Milan will hamper the cause of technical collaboration. Modest plans are now in order.

ALL the high hopes of the past several weeks that Europe would find a way of making itself technologically competitive with the rest of the world have been thrown into limbo. That is the simplest reading of the chaos into which the affairs of the European Communities have been plunged by last weekend's summit meeting at Milan. Formally, the heads of government did manage to agree that something should be done to put flesh on the bones of the French Eureka project; the French government is to call a meeting of foreign and research ministers in the second half of this month at which they will attempt to decide how the Eureka enterprise should work. The present plan is that governments other than the twelve members and putative members of the Communities should be free to participate if they wish. (Austria, Sweden and Switzerland are likely to be welcomed as outsiders.) The meeting is not intended to choose projects for collaboration but, rather, to define a mechanism which, governments agreed at Milan, should be above all flexible. The obvious snag is that while European governments are at loggerheads among themselves about the political future of their community, agreements on even technological collaboration are bound to seem unreal.

Ironically, the fiasco at Milan appears to have sprung from a sensible if belated recognition by European governments that the time has come to make what is often called the common market operate as such. Europe cannot be surprised that it is technologically often at a disadvantage in comparison with Japan and the United States when the present restraints on trade and competition persist. During the past few months, the European Commission has been hard at work on the definition of the steps that must be taken to dismantle these artificial and often illegal impediments to efficiency. Mercifully, all governments appear to agree that getting rid of these restrictions will be impossible while member states hang onto the principle that all decisions about the working of the Communities must be reached unanimously (or that any dissident member may veto an unpalatable proposal, as West Germany vetoed last month's agreement that cereal prices should be reduced). This is why the British went to Milan pushing for a package of procedural reforms that would have restricted a government's right to use the veto, and otherwise have made the community's decision-making more orderly. France and West Germany, on the other hand, with the unexpected backing of Italy, pushed instead for a revision of the Treaty of Rome, the legal instrument which is the foundation of the European Economic Community. The outcome is a fuzzy decision that, later in the year, there should be a formal conference to consider amendments to the treaty. This is a recipe both for delay and for further quarrelling within the community, if only because governments represented at this meeting will be able to veto any of the decisions it may reach. Especially because there is as yet no word on when or how the

conference will be organized, the upshot is bound to be that no framework for technological collaboration in Europe can be assured by the time of the Eureka meeting later this month.

Worse than that, the Milan meeting cannot but further sour relationships between European governments. Only occasionally in the past decade does it seem to have been possible for the heads of the European governments to meet without uncovering some obdurate disagreement. Last weekend's fracas at Milan will have further emphasized the degrees to which different members of the European Communities harbour different but serious reservations about the enterprise to which they belong. The European Communities have survived for the past thirty years chiefly because there has been a general recognition that attempts to force the pace towards European integration will be self-defeating if individual members are faced with politically unpalatable decisions. The attempt that will be made, later in the year, to force through amendments to the Treaty of Rome is bound to throw sand into the machinery of all kinds of European collaboration, Eureka included.

What can be salvaged from this mess? The best way forward for Eureka will be to postpone final decisions about the content of the programme of research and development on which Europe collectively seems eager to embark. In due course, it may emerge that there are technical projects of such originality that they promise opportunities for particular European industries to become genuinely competitive. Schemes for attempting to beat IBM at the game which it has played successfully over past decades should not, at this stage, qualify. It should also be clear that Eureka should not become one of those ambitious community projects in which there is some central pot of gold contributed by governments for a share of which companies in member states are encouraged to compete. The best strategy will be to use such public funds as there may be for supporting to provide the infrastructure from which novel technology may spring. Although President Mitterrand's clear wish (and political need) to clothe Eureka ambitiously would not be satisfied by, for example, public support for research and teaching institutions that could make good deficiencies in Europe, that may be the wisest course after the disaster at Milan. □

Where the onus lies

British science should make the government shoulder responsibility for political decisions.

ON the working hypothesis that the British government will not increase the funds earmarked for research in the next financial year (beginning in April 1986), researchers had better take what steps they can to save their own skins. In the next few months, the burden will fall chiefly on the Advisory Board for the Research Councils (ABRC), which technically exists to advise the Department of Education and Science on how the inadequate funds available should be divided. Two immediate issues must be settled. ABRC has to say how it would have the government respond to the report of the Kendrew Committee, which advocated a fortnight ago that Britain should pull out of European collaboration in high-energy physics (chiefly through CERN) unless there can be negotiated a 25 per cent reduction of the

Biological manuscripts

Contributors are reminded that, with the transfer of the Biological Sciences Editor to the Washington office, it will be helpful if they will in future send **four copies of all manuscripts** offered for publication, either (as at present) to London or Washington.

common budget after 1991. Then, unexpectedly, a committee of the Royal Society on public support for geophysics (see *Nature*, 27 June, p. 709) has unexpectedly thrown up a series of questions about the Natural Environment Research Council (NERC) which cannot be ignored until the council itself decides the time has come to answer them. What should ABRC say?

The Kendrew strategy is unhelpful for the simple reason that it will yield no budgetary relief when that is needed most, which is now. Even if savings of the order of 25 per cent are possible, which is improbable when CERN members such as Italy are eager to see high-energy physics in Europe expand, the first small relief for the British budget would come only in 1988. The difficulty into which the whole Kendrew exercise was boxed is that Britain is committed to the present scale of spending at CERN for at least the next three years by the undertakings given by the Science and Engineering Research Council (SERC) that there would be no backsliding while the new accelerator is being built, by similar undertakings to the international collaborations building detectors for the new instrument and by the implicit, but equally important, undertakings to graduate students whose careers are predicated on the completion of projects such as these. If Britain now follows Kendrew's advice, the course of events during the next two years is easily predicted. There will be a long wrangle at Geneva about future budgets, Britain will be blamed for rocking what has hitherto been a united enterprise, but in the end (if the strategy is kept to) will be forced to withdraw in humiliation. The political damage done will be no less than if it were decided to pull out of CERN immediately, moral obligations notwithstanding.

This dilemma is not of the research community's making, nor is it one that the community should on its own resolve. The difficulty has arisen because, while the British government refuses to recompense the research councils for the fall in the value of sterling except on an *ad hoc* basis, the CERN subscription (now £35 million a year) has become conspicuous. (No doubt it is only a matter of time before some other committee is appointed to enquire into the cost of the British contribution to the La Palma observatory, inaugurated only last weekend.) ABRC should now firmly throw this problem at the government, which did at the outset of the Kendrew inquiry say that it would have the final say on any proposal to withdraw from CERN. Now is the time for that. ABRC should warn the government of the political risks of pulling out, should explain that it cannot ask members of the scientific community to embark on a probably futile negotiation when the execution of the implied duress is beyond its own control, and meanwhile should insist that this and the other politically sensitive international subscriptions which are at present part of the science budget (such as the SERC subscription to the European Space Agency) are taken off the books of the research councils and handled centrally, by ABRC itself, with proper safeguards against currency fluctuations. Failing a satisfactory reply, ABRC should decline to follow the Kendrew strategy, but instead ask SERC to fight for economies at CERN, to cut back on what it spends domestically on high-energy physics but not to raise the threat of withdrawal until more is known of the future pattern of spending. More constructively, the question might be rather whether the CERN agreement should be amended. Is it strictly necessary that national subscriptions should be determined only by gross national product?

The problem of NERC is also urgent. The argument that this research council, cobbled together in the 1960s, should now be broken up is strong (see *Nature* 311, 493; 1984) but is probably unpalatable to too many influential people. Last week's Royal Society document was only incidentally an inquiry into the research council's way of working, and as such is not sufficient basis for root-and-branch reform. But a few things should be clear. First, it is absurd that Britain should pull out of the Deep Sea Drilling Program because NERC's funds are otherwise committed. ABRC should issue the appropriate instruction. Second, it is a tragic waste of people that the British Geological Survey's staff should this year lack the funds to do scientific work

for similar reasons. The cause may be the unwillingness of other government departments to pay for commissioned research, but the Royal Society's committee is right to say that the British national interest requires continuity in this field. If the survey's work can be secured only by transferring it to some other branch of the British government, so be it. Finally, given the committee's cogent criticisms of NERC's record on the award of research grants to academic geophysicists, there is a strong case for transferring that part of its function lock, stock and barrel to SERC. This is what ABRC should be saying, and quickly. □

Waxman's way

The US Congress should abandon its scheme to set up two new research institutes.

THE US congressional calendar is once again burdened by Representative Henry Waxman's attempt to wish on the National Institutes of Health (NIH) two entirely unnecessary research institutes, one for arthritis and one for nursing. The outcome is predictable, and will be no different from what it has been in the past three years. Waxman's bill, which has already been passed by the House of Representatives, will in due course be sent off to the White House and vetoed by the President. This is what happened last year, and nobody expects the outcome to be any different this time. The explanation is simple enough. The administration thinks the two extra institutes unnecessary, and has said so. It is also suspicious of Waxman's motives. Although this year's version of the bill (like last year's) is a considerable retreat from the earlier versions that would have given Congress detailed control of the way the individual institutes of NIH spend their budgets, there is no reason to think that Waxman has abandoned his ambitions to strengthen congressional control of the NIH budget. But while this legislation lies in limbo, three of the institutes (including the National Cancer Institute) will have to limp along from year to year by means of a continuing resolution that gives the administration authority for a year to keep on spending at last year's rate.

This is hardly the way in which the US Congress, which over the years has been generous almost to a fault to NIH, should deal with its favoured dependants. But it is not likely that, if NIH is required to set up further institutes, the flow of funds from Congress will be increased? That is how supporters of Waxman-like legislations have argued in the past, urging that NIH should welcome the creation of one institute for each recognizably distinct disease. It goes without saying that the political benefits for those members of Congress who force on NIH institutes dedicated to the cure of particular diseases will be far from unimportant. But this suggestion that a symbiotic relationship between Congress and NIH would ultimately benefit both is offensive because it betokens a thorough misunderstanding of the way in which NIH functions.

Congress, its constituency in the United States and biomedical research internationally have hugely benefited from the flexibility with which NIH are organized. In the recent past, the contribution of people at the National Cancer Institute to the understanding of Acquired Immune Deficiency Syndrome (AIDS) would have been no greater if Congress had decreed four years ago that there should be a special institute devoted to this novel scourge. Nor would matters have been much improved if, using the powers to control institute budgets in detail which featured in earlier versions of Waxman's bill, Congress had insisted that teams at the National Cancer Institute should not let their curiosity wander beyond the field of potentially oncogenic viruses so as to encompass that which now appears responsible for AIDS. In short, the recipe of one institute for one disease is a recipe for rigidity in a system where flexibility has miraculously flourished, sometimes against the odds. That is what Mr Waxman, and his colleagues in the Congress, should now openly recognize. □

Soviet scientist

Nuclear winter expert vanishes without trace

Stockholm

A SOVIET computer scientist, who has played a leading part in the nuclear winter debate, disappeared from a meeting in Toledo, Spain, last March, and is now feared by Western colleagues to be dead. Vladimir Aleksandrov was a Soviet delegate to SCOPE (the Scientific Committee on Problems of the Environment) and was an active participant in the SCOPE working party on the environmental effects of nuclear warfare.

Reports of last sightings of Aleksandrov vary, but according to one account he expressed his intention of going back to the meeting centre and was never seen again. According to another source, he was last seen being bundled into a car by several men. His personal effects were later found to have gone from his room, but his passport was found in a litter bin.

Former colleagues at the Lawrence Livermore Laboratories in California report frantic messages from Mrs Aleksandrov, who was greatly surprised and concerned at her husband's failure to return.

Aleksandrov was the author of the Soviet computer model using a two-layer model of atmospheric circulation which was presented in Stockholm in November 1983, and which supported the conclusions of Sagan and Ehrlich on the climatic aftermath of a nuclear war. Aleksandrov had allegedly told a Western colleague that he had been told to "get busy" on nuclear winter two months before the Sagan "peer review" meeting at the American Academy of Arts and Sciences in Boston in April 1983.

Aleksandrov had, during the past eighteen months, gradually been replaced as the chief Soviet scientific spokesperson on nuclear winter by Kiril Kondrat'ev, whose papers concentrated on nitrous oxides and trace gas effects, and paid little heed to smoke and dust. This may mean nothing more, however, than that once the computer model was complete, the Hydrometeorological Institute of the Soviet Academy took over the main burden of the nuclear winter campaign.

The silence of Aleksandrov's Western colleagues is difficult to explain. Some, however, seem to have thought that he had defected, and that the car incident was a cover-up. These have expected him to emerge from a safe house in some Western country in due course. But in that case, protest from the Soviet Union about what could well be interpreted as a kidnapping would have been expected, but none has been forthcoming. Nor have there been attempts to brand Aleksandrov

as a defector. One source which claims to be in contact with several Western intelligence services says that it is "not on the cards that the West has him".

If Aleksandrov was repatriated to the Soviet Union by force, as some of his friends now fear, and he was killed either deliberately or in the course of such an attempt, it is not clear what the motive

could have been. One suggestion is that he was feared to be a defection risk. Another is that Aleksandrov had become convinced that the Soviet concern with nuclear winter was merely for public and international opinion and that the Soviet military planners were working on the basis of a "survivable level" of fall-out, and that he intended to voice these views.

Such suggestions, perhaps farfetched, reveal the growing concern about his fate. Hopefully, however, even at this late date, the Soviet Union or some Western government will respond with some explanation and, if possible, the production of Aleksandrov in person.

Vera Rich

Nuclear power

US plans to set industry free

Washington

ATTEMPTS are again being made to revitalize the moribund US nuclear power industry by speeding up the licensing of new plants. Three bills* now before Congress, from the Department of Energy, the Nuclear Regulatory Commission (NRC) and from the industry itself, seek to encourage the use of standard plant designs by giving NRC authority to issue combined construction and operating licences (COLs) at the design stage. At present, NRC must hold a second examination of safety issues before a completed plant can be operated.

The argument is that by forcing all safety issues into a single examination, NRC will give utilities an incentive to perfect complete designs that can be adapted to the requirements of different sites. Construction permits have often been issued for incomplete plans, an approach characterized by the Union of Concerned Scientists (UCS) as "design-as-you-go", resulting in frequent expensive modifications.

There is widespread agreement that the present two-step licensing procedure is inefficient, and that engineering expertise would be better used if there were but a few standard plant designs to be examined. But opponents of the new proposals argue that they fail to encourage standard designs, and that they make it harder to raise serious safety problems once a plant has been built.

Each of the bills recognizes that some kind of pre-operational safety review is necessary before operations can begin, but to prevent determined opponents of a plant from raising questions of safety that have been adequately considered at the design stage, each contains some restriction on the issues that can be raised in a pre-operational review. The NRC bill, for example, says a new issue must be a substantial dispute of fact that cannot be resolved except at a hearing, and that it must either concern non-compliance with a previously-issued COL or a question that

could not previously have been examined. Controversially, the NRC bill also says that for a post-construction modification to be required, it must substantially improve the overall safety of the facility. Overall safety is examined using probabilistic risk assessment, which according to its critics is nothing better than an obfuscatory device that, by being easily manipulable, is used to give spurious authority to safety claims. The NRC bill also raises hackles because it would allow a plant to begin operating before hearings end.

The Department of Energy's bill would make it even harder for safety issues to be introduced retrospectively, as it would allow NRC to make conclusive design approvals that would remain in force for 10 years no matter what information was subsequently discovered; furthermore, it would allow the licensee to deviate from the design in the COL as the licensee saw fit. The industry bill gives intervenors greater latitude to introduce safety issues at a late stage, but requires NRC to perform a central review before incorporating changed requirements. This could mean delays of years.

Opposition to the three bills, voiced more articulately by UCS, focuses on the argument that NRC already has the legal authority to approve standardized plant designs. UCS says it is the fault of the industry that such designs have not been used in the past, because it has sought construction permits on the basis of an examination of very incomplete plans. According to UCS, the depressed state of the industry — no new plants ordered since 1978 — would change if NRC insisted on seeing a complete design before granting construction approval. Industry spokesmen contritely acknowledge that approval of incomplete plans is counter-productive and promise that utilities will submit "essentially complete" plans in future.

Tim Beardsley

*HR 1029 (from the industry) and HR 1447 (from NRC); the Department of Energy bill has not yet formally been introduced in the 99th Congress.

Biotechnology

Variety at *Nature* conference

PLANTS engineered to produce antibodies against viral pathogens, protein frameworks into which various of functional units can be plugged at will and biosensors based on a marriage of immunology and fibre optics were among the more engaging ideas to emerge from *Nature's* "New Technology in Biotechnology" conference at London's Novotel last week.

Clearly the path to commercial reality from an engaging biotechnological idea is long and exceedingly stony, but it is increasingly travelled. Ten years after the discovery of hybridoma technology, John Birch could talk of the production at Celltech of around a kilogram of monoclonal antibody a year, mostly for blood group testing, (see *Nature* 27 June, p. 705) and its sale for, typically, £1,000 a gram.

Clever variations on the basic theme of monoclonal antibodies are already proving their worth at the Molecular Research Council's Laboratory of Medical Biology in Cambridge. Most are the result of engineering the genes for antibodies, replacing an inessential part of the sequence with another gene. The results are hybrid, bifunctional proteins, several of which were described by Michael Neuberger. In one, the substitute gene is for an enzyme used in DNA sequencing. After the hybrid antibody/enzyme is produced in large quantities by standard hybridoma technology, it is very simply and efficiently immunopurified by means of its antibody end; the other end is now supplying the laboratory's considerable demand for the enzyme. On the other side of the coin, a protein that is unable to induce good monoclonal antibodies has been engineered to contain an extra short sequence so that it can be recognized by an existing and excellent monoclonal antibody against the short sequence.

Daniel Thomas of the University of Compiègne foresaw the value of hybrid enzymes in second-generation immobilized enzyme technology. For example, the lifetime of an oxidase that is susceptible to damage by some of the oxygen radicals it produces could be lengthened if the ox-

idase were to be hybridized with a superoxide dismutase, which can consume the radicals. Other important second generation systems would, for example, have built-in systems of co-factor regeneration on electrochemical surfaces or would operate in organic solvents, perhaps to reverse an enzyme reaction or in organic synthesis. More sophisticated protein engineering will involve the subtle redesign of active sites of enzymes, which will remain very much a matter of trial and error until much more is known of the determinants of protein folding, according to Greg Winter of the Medical Research Council Laboratory of Molecular Biology in Cambridge.



R. Brinster

Even without engineering, enzymes and antibodies have many projected uses in biosensors, very few of which have yet progressed from the laboratory to the market place. Michael Flanagan of University College London described prototype optical immunosensors in which the binding of fluorophore-labelled antigen to antibodies immobilized on a surface of the device is measured by means of evanescent light waves of plasmon resonance laser light. For potentiometric devices, enzyme immobilization techniques badly need adapting to suit the surface of microelectronic devices, he said. For amperometric devices, John Higgins of Cranfield Institute of Technology and Leicester Biocentre described current interest both in the organometallic ferrocenes as mediators of electron transfer to electrodes from enzymes bound to them and in a newly discovered class of dehydrogenase enzymes that use pyrroloquinolinequinone as a cofactor in place of the more usual but problematic nicotinamide adenine dinucleotide.

Recombinant DNA technology lags behind that of hybridomas and perhaps even biosensors in its commercialization. But there are products in the offing. Pierre Tiollais of the Pasteur Institute described the advanced state of a hepatitis B vaccine from engineered mammalian cells and Art



J. Schell

Levinson of Genentech claimed that the company's tissue plasminogen activator has been able to dissolve blood clots and restore blood flow through the coronary arteries of some patients in a clinical trial.

For plant breeders, the most immediate gain is likely to be strains that have been engineered to be herbicide resistant, according to Jozeph Schell of Ghent University and the Max Planck Institute for Plant Breeding Research in Köln. Less advanced are attempts to improve potato protein quality and quantity, to transfer light-inducibility to new genes and to engineer cereal crops. And but a glint in Schell's eye is a plan to give tobacco plants the mammalian gene necessary to produce a protective antibody against tobacco mosaic virus. For animal breeders, Ralph Brinster of the University of Pennsylvania offered encouraging news of the general applicability of the introduction of genes into fertilized eggs and the tissue-specific expression of the genes at high levels in the subsequent transgenic animals. One recently learnt fact is that the removal of residual bacterial sequences from a gene construction introduced into the eggs can greatly increase the gene's expression in the tissues of the transgenic animals.

Peter Newmark

● For those who would like a record of the *Nature* conference, cassette tapes are available from: Audio Bass Ltd, 16 Longland Drive, Totteridge, London N20 8HE, UK (Tel: 01-445 6776 or 01-349 4041). Please quote programme number 064-85.

● The correct answers in the exhibition competition were as follows:

1. Tissue culture bottle/Laminar flow hood
2. Eppendorf microfuge
3. Dilon pipetman
4. Scintillation counter
5. Tube sealer
6. Fibre optics

None of the entrants identified all the photographs correctly. Sally Roberts and Pam Jones, both of the Laboratory of Molecular Biophysics at Oxford, got five out of the six right, and in the draw Sally Roberts was selected as the winner of the personal computer. □



P. Tiollais

French education

Higher marks for effort

ONLY 37 per cent of French children attain the coveted *baccalauréat* (*le bac* for short) which guarantees entry to university. But the figure should be 80 per cent by the end of the century, according to the French minister of national education, M. Jean-Pierre Chevènement. Such an increase would raise the number of students in higher education in France to 2 million, four times the figure in Britain, which has a population of the same size.

If all 2 million students were to proceed to higher education, the 80 or so French universities could not cope. At present, with just one million students, as many as two-thirds of entrants leave university after the first year, without a diploma. (Lecturers dub them "ghost students" — some do not even attend courses.) In practice, the ministry of education expects that only a fifth of the extra million French students, who will come through a new form of baccalaureate, *le bac professionnel*, will be seeking places in universities or the French technical colleges, the *Instituts Universitaires de Technologie* (IUT), which are sought after by parents and students because education there usually results in a job.

Others may go to new universities of technology (there is only one at present in France, at Compiègne) or to an "open university", both of which have been promised in recent weeks by the French government. But most of those attaining the new *bac professionnel* are expected to go straight into employment.

The new scheme is designed to loosen up the less academic and employment-oriented French high schools, to make them adapt their education to the real needs and demands of local employers, and to extend the extra 2–3 years of secondary education required for *le bac* (the examination is usually taken at the age of 18) to a wider social group.

The new move must also be seen in its true context as part of the phenomenon of the minister himself. Chevènement is an old-fashioned socialist working in the 1980s. He believes in equality of opportunity: the new *bac* will provide that. He believes in industry: the new *bac* will be tuned to it. But secondary school students will have to work. Chevènement believes in attainment. He also believes in science, as he believes passionately that socialism is equated with knowledge. As President François Mitterrand's first research minister, Chevènement reversed the anti-industry attitudes of most of the French research community, which may be the most substantial achievement of the socialist administration in its four years in power. And now, after a period in political limbo, he seeks to make further lasting marks on France by reshaping the whole education system.

Out have gone the "advanced" liberal attitudes of the 1968 student revolution, whose anti-authoritarian spirit affected schools almost as much as universities, and in has come what the minister calls "republican elitism", basically a renewed respect for an encouragement of academic attainment and for the republican ideals of post-revolutionary France.

"A structured school is more democratic than a lax school", Chevènement wrote recently, discussing the nature of his "elitism". In a lax school, the more confident and middle class pupils rise to the top of the heap. In a structured school, native ability wins through. The proportion of

working class children at universities is still very low, and the minister intends to change that by providing academic and professional opportunities. Hence the new *bac*, which will also have the advantage of helping to train the kind of technicians that new industries need.

Among other important innovations, Chevènement is working on reducing the power of the mathematicians in French schools, which is thought to discourage experimental and observational sciences, in particular biology and geology.

Chevènement has already outlined his ambitions for the universities (see *Nature* 9 May, p.88) but he may not have long to achieve his aims. There will be a general election next year, and the polls suggest that the socialists will be defeated.

Robert Walgate

European computing

Toulouse carries ambitious torch

THE FRENCH proposal for a European computing centre at Toulouse in south-west France, described by a British computer scientist as "a kind of CERN [the European Organisation for Nuclear Research] for computing", has met at least with the approval of Italy. But Britain appears sceptical, while in Toulouse itself, hopes were high for support from West Germany.

That was the position last week over the future of CERFACS, the "Centre Européen de Recherche et de Formation Avancée en Calcul Scientifique", an £18-million-a-year* institute for research and training in numerical analysis whose establishment has been championed for over two years now by a group of Toulouse astrophysicists, aerospace engineers and politicians. According to CERFACS supporters, existing techniques of numerical analysis are out-of-date, having been designed for machines with a single central processing unit. With new computer architectures involving vectorial and, increasingly, parallel processing, where the same or different functions can be performed many times simultaneously, numerical analysis needs to be rethought. CERFACS would do the rethinking, provide training and help apply the techniques to develop areas such as the creation of numerical "wind tunnels" (flight testing by pure hydrodynamical computation).

According to CERFACS supporters, with an eye on the 1986 French budget as it approaches its final month of inter-ministerial bargaining, apart from possible support from Germany and Italy, the Netherlands, Denmark and Switzerland are also ready to join in, provided a second country (other than France) offers support. And in France, while government support is still not guaranteed, there is a chance that CERFACS may be caught up in the political snowball of "Eureka"

(see pages 8 and 9).

But there is some concern in Paris, and even more in London, that CERFACS is too ambitious and costly, and should be prepared to grow "at a natural pace". There are "a lot of traps" in the Toulouse plans for CERFACS to start up in September 1987 with over 100 staff, rising to nearly 200 by 1991, it is said in Paris. In London there is a feeling that the centre would not offer "value for money".

Professor Jean Yoccoz, the director of the French nuclear and particle physics institute IN₂P₃, however, says "there are always pluses and minuses" and that "the best of CERFACS" is its plan to mix industrial and fundamental research on one site, as well as training. And Toulouse, with its aerospace and robotics industry, "is a good place for that, at least for France".

A leading British computer scientist, however, is less impressed. He feels the emphasis on new numerical and software techniques for use with vectorial supercomputers is "very esoteric" and "not leading edge computing science".

In Toulouse, however, these criticisms have been met with "surprise". CERFACS is prepared to deal with whatever computer technology is in the forefront, be it a vectorial Cray 2, or the German multiprocessor Suprenum, or a machine still on the drawing board. Far from being esoteric, CERFACS — or something like it — would be essential to the proper use in Europe of these new machines, it is claimed in Toulouse and whatever equipment was provided would moreover be networked to users throughout Europe. "We aim to respond to user needs", a spokesman says. And although the planned rate of development of CERFACS is indeed ambitious, Europe cannot afford to wait to be pre-empted in yet another area by the United States and Japan.

Robert Walgate

Deep-sea drilling

Britain squeezed onto margins

Washington

STRAINS are beginning to show over Britain's continued inability to find the \$2.5 million membership fee needed to join the Ocean Drilling Program (ODP). British scientists have been removed from all planning and advisory committees of the programme, and according to Robert Kidd, manager of scientific operations of the research vessel *Joides Resolution*, British scientists are unlikely to be invited to participate in future cruises.

Britain at present enjoys observer status in ODP, which permits it only to send an observer to meetings of the executive committee of Joint Oceanographic Institutions Deep Earth Sampling (JOIDES). Japan, which also has observer status, recently signed a memorandum of understanding with the United States saying that it will join the programme as a full member at the start of October this year; no such guarantee has yet been made by Britain. As a result, the only British scientist on the current cruise of *Joides Resolution* in the Norwegian Sea, Lindsay Parsons of the Institute of Oceanographic Sciences, is likely to be the last until Britain rejoins, unless there is a cruise in British territorial waters.

Planners at ODP find themselves in a political bind: although they want to invite individual British scientists because of their expertise in particular fields, it is felt that it would be unwise to send the British government a signal that its best scientists will be able to participate in ODP whether or not Britain becomes a member. The assistant programme director of ODP at the US National Science Foundation, Alexander Sutherland, says JOIDES has been "pretty adamant" that British scientists should not be offered guaranteed places, out of fairness to participating countries. Dr Parsons' participation in the current cruise, leg 104, was arranged before the JOIDES committee forbade guaranteed places for British scientists, and was supported (despite misgivings in some quarters) by the two co-chief scientists on the cruise, Olav Eldholm of the University of Oslo and Jorn Thiede of the University of Kiel.

Britain's difficulties in finding the necessary money has particularly annoyed Canada, which has paid despite having a much smaller gross national product. Although the European Science Foundation and Australia may join ODP as a consortium, JOIDES has ruled out the possibility of Britain joining as part of a consortium.

Britain's Natural Environment Research Council still hopes to be able to raise the necessary funds in time, although many people in the United States seem resigned to the fact that Britain will not be a member next year, at least. One political

factor that may yet carry the day in Britain, however, is a planned cruise in the Weddell Sea: a scientific interest and

presence in Antarctica would undoubtedly bolster British negotiating positions when the Antarctic Treaty comes up for renegotiation at the end of the decade. Only by being in at the planning stages will Britain be able to influence the shape of the cruise.

Tim Beardsley

Auto emissions

Europe agrees at last

Brussels

AFTER an all-night session, environment ministers of the European Economic Community (EEC) came to the close of their meeting in Luxembourg on 28 June just in time for breakfast with agreement on new tougher European exhaust emission controls, the first of which will be applicable from 1988.

The deadlock over this controversial issue, which has kept the environment ministers busy over one aspect or another for two years, was overcome by a decision not to follow the US example, despite pressure from West Germany, to quantify the exhaust emission limit for nitrogen oxides (NOX) on their own for cars in the intermediate range of 1.4 to 2.0 litres.

The combined figure for nitrogen oxides and hydrocarbons (HC) has been common practice until now in the regular modifications made to the 1970 EEC directive on exhaust emissions, and gives motor manufacturers room for manoeuvre within a range of engineering options. In getting to grips with emissions, one of the problems in engine design is that modifications to the combustion process aimed at reducing, for example, hydrocarbon emissions increase the production of NOX and *vice versa*.

The limit finally agreed — 8 gram/test for HC and NOX together, the European Commission's proposal — falls half-way between what West Germany wanted on the one hand and what the British were prepared originally to accept on the other.

For the United Kingdom, the difficulty lay in accepting a figure that it might be impossible to obtain using lean-burn single-point ignition, under development in the United Kingdom. Furthermore, the use of this technology (lean-burn plus oxidation catalyst) had been part of the agreement hammered out by environment ministers at their previous meeting in March. As it turned out, Mr William Waldegrave, Under Secretary of State at the UK Department of the Environment, agreed on his own responsibility to the

figure pending a government decision in London. No hitches are expected.

This spirit of European compromise has meant less of a warm welcome from environmentalists in West Germany for their interior minister Friedrich Zimmermann, for whom coherence in the Community's external market, at the end of the day, outweighed tough political pressure on a national level.

Ministers also accepted a number of other ancillary points, said to have been initially proposed by French environment minister Huguette Bouchardeau, which aim at further reducing exhaust pollution (and tension between the member states):

- That small cars (under 1.4 litre) would have to conform earlier to stricter standards, bringing them in line with US environmental equivalent effects, by 1992 for new models and 1993 for all new cars.
- That the United Kingdom and France would agree to drop complaints made to the European Commission against the national fiscal incentives for "clean cars" announced by West Germany at a time when European legislation on the subject was being prepared (illegal under Community law).
- That the Commission should propose legislation in the next three months to limit emissions from lorries, and in particular on diesel particulates (see *Nature*, 311, 401; 1984). Ministers also laid down new standards for diesel engines with a capacity of 2 litres and over. While, in the past, limits for diesel engines have had the advantage of a 25 per cent waiver in their favour, they will in the future have to comply with the same new limits established for the intermediate range of conventional car engines.

● That the Commission should make proposals on speed limits and on a regular monitoring system on vehicle compliance, possibly based on the tough system applied in Belgium.

In addition, the Commission itself may decide to propose further measures.

Anna Lubinska

	Emission standards		Deadline (1 October)	
	CO	HC + NOX	New models	New cars
Cars over 2 litres	25	6.5 (3.5 NOX)	1988	1989
Cars of 1.4-2 litres	30	8.0	1991	1993
Cars below 1.4 litres	45	15.0 (6.0 NOX)	1990	1991

(Stage 1)

(Stage 2 to be agreed in 1987 with implementation by 1992-93)

Japanese education

Council's criticisms start row

Tokyo

JAPAN's special Council on Education, set up to prepare a blueprint for a massive reform of the education system, delivered its first set of recommendations last week to a chorus of complaints and disapproval.

It is only ten months since the council, with its 25 members all personally picked by Prime Minister Yasuhiro Nakasone, began its activities, so it is perhaps not surprising that it has been unable to reconcile all the fiercely held views on this emotive subject. But the report is more of a philosophical declaration than a practical recipe for reform.

The report castigates the present education system in terms which, if they had been written by a foreigner, would probably have given rise to complaints of anti-Japanese prejudice. The system is accused of producing "too many stereotyped persons without strong individuality" who are not "able to think and judge on their own" because they have been taught only to "memorize information and facts". "Bullying in school, pupils' absenteeism, violence at school and juvenile delinquency" are also described as "grave" and as products of the present system, although Japanese schools are havens of peace compared with those in the West.

To remedy this situation, strong emphasis is placed on the fostering of "individuality". But although this Western notion is repeatedly endorsed, far more column inches of the report are given over to the need to reinforce distinctively Japanese values: children "must love Japan", maintain "a deep understanding and love of Japanese culture" and have "identity as Japanese". These values were, of course, inculcated in the education system that existed before the Second World War, which, indeed, comes in for some oblique praise, while post-war education is criticized for putting "a disproportionate emphasis on rights instead of duties". To the Socialist and Communist parties (the second and fourth largest parties in the Diet) such statements are like a red rag to a bull and they have attacked the report furiously, pointing out the difficulties in a system which is meant to teach people to "think independently" while "fostering Japanese who will inherit the traditional culture".

Philosophical contradictions aside, the report is firm in identifying the real villain behind educational woes: a society that gives too much preference to graduates from leading universities. Because top corporations and ministries recruit from just a few universities, the whole educational system has become oriented towards success in the university entrance examination "hell". In that examination, all that counts is overall score in multiple choice questions; the higher one's score

the better the university (or faculty) one can enter, regardless of vocation and aptitude. The report makes an appeal for a change in attitudes away from a society oriented towards "academic record", but clearly such changes are beyond its power to effect.

A more direct attack is made on the university entrance examination. A new first-stage unitary examination is proposed for use by all universities, public and private, to replace that now used only by public universities. The burden on students, who usually try more than one kind of examination, would thus be eased.

To try to ease competition further down the system, new kinds of schools are proposed that will fuse junior and senior

high-school. Entrance examinations for high-schools have recently become almost as fraught as the university entrance examinations themselves. It is suggested that some such schools work on a credit system for high-school graduation rather than rely on intensive examinations to increase the flexibility of the system.

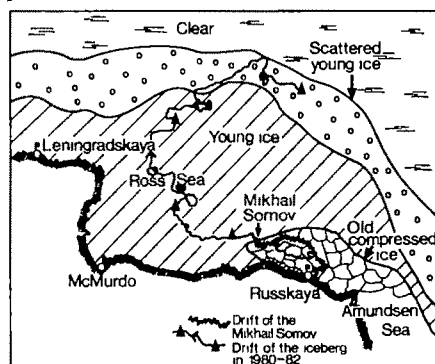
The Council on Education will now soldier on for a further two years, trying to build on this somewhat shaky start. The Prime Minister is giving full backing to the council, and a cabinet-level group is expected to be formed to put its weight behind its recommendations. So far, however, teachers are opposed, the Japan Teachers Union having sworn to fight the reforms "to the end" a few days before the report was even published. The teachers' main concern is to get average class sizes down to 35 from present levels which are closer to 40 pupils.

Alun Anderson

Soviet Antarctica

Trapped vessel in good shape

THE Soviet research vessel *Mikhail Somov*, which has been trapped in the Antarctic pack-ice for more than three months, could eventually drift to safety along a route mapped by a previous expedition aboard the same vessel. Accord-



ing to Candidate of Sciences Al'bert A. Romanov, of the Arctic and Antarctic Research in Leningrad, the ship's present course virtually coincides with that of an iceberg, mapped by radio beacon as part of a Franco-Soviet experiment in 1980-82. The tagged berg drifted on a semi-circular course for more than 3,400 nautical miles before emerging into the warm waters of the Pacific.

The planners of the 30th Soviet Antarctic expedition, of which *Mikhail Somov* is the flagship, do not, however, intend to leave the crew and research personnel trapped for so long. Some 77 people have already been flown out by helicopter, leaving 53 on board for essential ship maintenance and observations of conditions in this previously unexplored sector of the Antarctic. (Conditions are severe, with winds of 17 metres per second, temperatures of -25°C , poor visibility, snowstorms and fog.) Meanwhile, a special taskforce has been established under the deputy head of the state committee for

hydrometeorology and environmental control to "ensure a rapid solution" of the "*Somov* problem". The icebreaker *Vladivostok* of the Far Eastern Shipping Line has been sent south, and is expected to approach *Mikhail Somov* at the beginning of July.

Because *Mikhail Somov*, with its now reduced complement, is well provisioned for another 10 months, evacuation plans are, so far, of a contingency nature only. A specially adapted Mi-8 helicopter is on board the *Vladivostok* to evacuate the *Somov* personnel in case of emergency.

For the moment, *Vladivostok's* chief role will be to try to break a channel through to the trapped vessel and, if necessary, tow it out. If *Somov* could break through into the relatively young ice of the Ross Sea, the most difficult part of the mission would be over. Meanwhile, scientists are continuing their work aboard the trapped vessel, including, presumably, the seismo-acoustic probing of the sea-bed that was a major feature of the ship's original programme. It is unfortunate that Soviet plans to have all seven permanent Antarctic stations equipped with Inmarsat receiving and transmitting equipment have not yet been implemented. So far only one such station, the Molodezhnaya observatory in Enderby Land, is so equipped, while the two nearest to the trapped ship, Russkaya and Leningradskaya, are linked to Leningrad only by shortwave radio.

Vera Rich

Correction

There was an error in last week's story about laser fusion (*Nature* 27 June, p.706): the current budget for inertial confinement fusion is about \$140 million; the administration proposed halving this sum in the fiscal year 1986.

Eureka

Brussels hope after Milan chaos

Brussels

WITH the key issue of institutional reform referred, reluctantly by some, to an inter-governmental conference to take place later this year, the approval by heads of government of the European Economic Community (EEC) of the idea to give the Community a new technological dimension may be considered as, at least, one positive result at the end of the two-day summit in Milan. But a series of questions still remain unanswered.

EEC heads agreed that technology in the Community should be strengthened. Thus they agreed that there should be a close link between technological development and the effort to unify the internal market, that technological effort should be closely tied to common policies as on trade policy, that duplication of national efforts should be reduced and money pooled.

The Milan summit also endorsed a Community report on the subject but the heads of government gave no clues as to the form research collaboration would take and who would coordinate it.

Questions of financial support and structure will be considered at an *ad hoc* meeting on European technology by EEC research ministers and "other qualified representatives of governments" (which may imply industrial participation). Government heads asked the French (perhaps not insignificantly) to convene a meeting in Paris, probably on 17 July. These "technology assizes" will also include the new (from 1986) EEC members Spain and Portugal, as well as non-EEC countries Austria, Sweden, Switzerland and Norway to whom participation in projects will be open and who have expressed an interest. At Milan, two proposals were put forward, the Eureka project in which countries and companies may collaborate unilaterally or in groups according to the dictates of interest and investment capacity, and the Commission proposal which would like to see the Eureka project as part of a flexible structure coordinated on a Community level, with projects operating on the lines of the Joint European Torus at Culham in the United Kingdom.

The Commission proposal has the advantage of being able to fall back on existing structures, and it has the support of the smaller member states which need to share the financial burden of technological research investment. EEC research commissioner Karl-Heinz Narjes has suggested the creation of a corridor in the research budget as well as increasing the share of the budget itself.

The other possibility is that Eureka might develop separately, out of reach of Community control (which would please President Mitterrand) but which would be overseen by an intergovernmental agency

which would at the same time keep an eye on the Community programme and would thus coordinate the two. A possible candidate for the job is Viscount Etienne Davignon, ex-EEC research and industry commissioner and father of the much quoted ESPRIT programme which launched cooperation between European industry and universities. In this second case the ten Community countries would be treated as just another member state in

Eureka

Companies ready to bid for funds

WHILE European heads of state have been debating whether and how to go ahead with Eureka, the French proposal for a joint European technology programme, electronics companies in Europe have been defining projects that their political masters might support. Last week GEC (United Kingdom), Siemens (West Germany), Philips (The Netherlands) and Thompson (France) announced that they have already set up a study group to make joint proposals to Eureka, while the Norwegian computer company Norsk Data and the French defence, space and electronics group, Matra, announced the development of a super-mini-computer, a "Crayette" (for scientific use) under the Eureka banner.

These are some of the first concrete developments to flow from the Eureka idea originally proposed by President Mitterrand of France to provide Europe with an alternative to participation in the US star wars programme. The speed with which they have been put in train would seem to indicate that Eureka has touched a receptive nerve.

According to British GEC, experience of the European Commission's ESPRIT programme for pre-competitive research in information technology, while positive, has indicated the need for more market-oriented cooperation in real product development, which might be stimulated by Eureka. ESPRIT itself has "barely started", but has shown that an "unbiased" organization like the Commission can catalyse cooperation, spanning national frontiers.

A further factor is money. While nothing has been agreed, French sources have suggested that France alone would be prepared to contribute an initial £100 million to Eureka. Development costs in electronics seem to be increasing exponentially, according to Philips (Eindhoven) directors, so that the potential availability of public pots of gold is stimulating. International cooperation in development would also ease the burden.

What products are in the offing? Derek

participating in Eureka projects.

Conceding that Eureka should develop separately would allow at least some member states to get going on technological research while the institutional debate in the EEC continues. For even if the Commission maintains that the proposal is based on existing operational rules, bringing about a "qualitative technological leap forward" will require, as Jacques Delors, president of the European Commission, insisted before the Milan summit, changes to inject dynamism into functioning of community institutions and encourage the creation of an internal market.

Anna Lubinska

Roberts, technical director of GEC, appears to have in mind new transportation systems and traffic control systems, while pan-European cellular radio networks are also in the air. The French have also been pushing for the development of a large European super-computer to rival the Cray machines.

Meanwhile, Norsk Data, which first made its name by supplying the computer control systems for the super proton synchrotron at CERN, and Matra are working together on a more immediate project, to develop not a European Cray, but something that might be described as a "Crayette".

The two companies have already been cooperating on the development of a 32-bit mini-computer to supplant Digital Equipment's ubiquitous VAX and, says Matra, they have identified a market gap for desk-top vectorial machines. These, like the Cray, offer a degree of parallel processing, essentially performing instant matrix multiplications on columns of numbers ("vectors") many elements long. Such computers are proving essential in number-crunching operations as in quantum chemistry, atmospheric modelling and seismology, but they are at present very expensive, ranging from £1 million to £10 million.

Norsk Data and Matra, however, hope to come on the market (with the help of Eureka) in two years with a vectorial machine of some "hundreds of millions of operations per second" at a price of only £100,000 to £1 million, compared with the current price of a VAX of around £60,000. Matra is aiming at the market for 32-bit minis in 3-4 years time.

One Cambridge quantum chemist said last week that "there's no doubt that this is the way it'll go" and that the Matra/Norsk Data proposal sounded "extremely competitive". The present cost of vectorial computing is a real hindrance to its development, he said. Companies such as Convex of the United States would be in competition with the European group, however.

Robert Walgate

Bioethics

US Congress tries again

Washington

THE US Congress is trying to fill the gap left by the 1982 demise of the President's Commission on Bioethics. Last year's attempt failed when President Reagan vetoed the National Institutes of Health (NIH) authorization act, which included provisions for a panel to monitor human genetic engineering, protection of human research subjects and other issues in medical ethics.

Last month, the House of Representatives again passed the NIH bill proposed by Representative Henry Waxman (Democrat, California), largely unchanged from last year's version. A similar bill is pending in the Senate. But its fate must be uncertain. Both House and Senate bills retain the features its president found objectionable last year, in particular the detailed specification of each NIH institute's research mission labelled, in the veto message, as "micromanagement" by Congress. Both

bills also call for two new NIH institutes, one for arthritis and one for nursing. NIH has long opposed the creation of new institutes, which it says add to its administrative costs without augmenting or improving the research.

Even if there is a veto, the bioethics panel may survive. Senator Albert Gore (Democrat, Tennessee), a key supporter of the concept when he was in the House, has filed a separate measure much along the lines of the section of the House and Senate bills dealing with the panel. A bipartisan board would appoint a committee of biomedical researchers, physicians, ethicists and laymen to carry out studies of ethical problems in biomedicine. The committee would have no regulatory function and would operate as an agency of the legislative branch, akin to the Office of Technology Assessment and the General Accounting Office. There is a strong feeling among even supporters of human gene therapy that a high-level but nonregulatory panel would be the best way to evolve policy on its new medical technologies while disarming the more extremist critics. A less-favoured alternative is an expansion of NIH's Recombinant DNA Advisory Committee (RAC) which has begun to draft guidelines for human gene therapy experiments. But RAC, as part of an executive branch agency, also lacks the formal independence that many would like for an ethics panel.

Another role of the ethics panel would be to straighten out the muddle over fetal research. Under current rules, NIH may not support research posing "greater than minimal" or "unknown" risks to a fetus. Although the Secretary of Health and Human Services (HHS) is allowed to grant a waiver if an experiment has passed the scrutiny of the local Institutional Review Board, the advisory council of the relevant NIH institute and a specially empanelled Ethical Advisory Board, the Secretary of HHS has never been willing to appoint an Ethical Advisory Board. In effect, there is a total moratorium.

Under the House and Senate NIH bills, the ethics panel would be asked to study the question of experiments involving unknown or greater than minimal risks. Waxman said he had reluctantly agreed to go along with the Senate's insistence on a 3-year moratorium even on waivers in the hope that this would allow time while "tempers cool and rhetoric dies down". He noted, for example, that a study on the dangers of vaccinating pregnant women against rubella, which was carried out in 1969, before the current rules became effective, would probably now require a waiver. In that study, women planning to have therapeutic abortions were vaccinated, after the abortions took place

11 to 30 days later, fetal tissue was examined to determine if the vaccine virus was able to cross the placenta and infect the fetus. The answer, contrary to expectations from animal studies, was yes; and physicians were ordered not to administer the vaccine to pregnant women.

As a practical matter, however, the 3-year moratorium will not affect research, and Waxman said he hoped that after a "full and even-handed" review by the ethics panel, the Secretary of HHS would "proceed with the responsibilities to pursue research ethically and without regard to purely political pressures".

Stephen Budiansky

Ethics in science

Washington

ONE of tenets of the scientific method is that researchers are supposed to share their data with others who wish to verify or expand on their work. That that apparently does not always happen is the unstated but clear implication of a new report from the National Academy of Sciences* urging the adoption of guidelines by grant-making agencies and scientific journals that will normally require data-sharing.

The report recites the usual reasons for sharing data: encouraging open scientific inquiry; allowing for verification, refutation or refinement of original results; bringing multiple perspectives to bear; encouraging interdisciplinary use of data; and detecting the rare cases of fraud.

But, the report notes, researchers may be reluctant to hand over their data to other researchers for various reasons.

The report recommends that researchers make their data available by the time of publication of the initial major results: "the practice of withholding data until all possible analyses are exhausted is unnecessarily restrictive and too self-serving to advance science", it said. Those who request data should bear any incremental costs, however.

Government funding agencies, the report says, should require applicants to guarantee data-sharing or to justify explicitly, in their grant proposals, why they should be exempt from the requirement. The National Science Foundation (NSF), for example, already requires that "data banks or software" produced with NSF grant funds be made available to others; the report notes, however, that the policy could be strengthened.

Finally, the report recommends that scientific journals should require authors to provide peer reviewers with access to their original data and "strongly encourage" them to provide similar access to others, and should give more space to reports of secondary analyses and replications to encourage the process.

Stephen Budiansky

*Sharing Research Data, Committee on National Statistics, National Academy Press, 1985

Paris scents triumph

Paris

IN France, the feeling over Saturday's summit debacle over the European Community's Treaty of Rome appears to be one of sweet revenge over that De Gaulle of an Englishwoman, Mrs Margaret Thatcher, and for the years of confrontation over the British budget in Brussels. But the sweetness will certainly turn sour if the result is to damage the French-inspired Eureka project for European technology.

So far, however, all seems well. The summit has produced an agreement to hold here in Paris in the second half of this month a "technology conference" of European foreign and research ministers which should, at last, result in the definition of exactly what Eureka will be, who will run it and who is prepared to pay. But the fear among those closest to Eureka in Paris may be that the conference will be compromised by the arguments among their political masters in Europe over other issues.

The French sources were at pains on Monday to distance Eureka from the battle. "Mrs Thatcher showed herself quite constructive on technology", a French research minister spokesman said, and the split over veto rights and the Treaty of Rome was "quite another question".

The apparent conflict in Milan between François Mitterrand and the president of the European Commission, Jacques Delors, was also being played down in Paris this week. The view here is that the European Commission can be "closely involved" and that "Eureka is not in conflict with the Commission". The communiqué from Milan "took note of and endorsed" Delors' plan. Robert Walgate

A capital Universe?

SIR — On one side of the Atlantic, authors and editors continue to use the word universe, whereas on the other they have changed over to the word Universe. Last year, while hastily proofreading an article written for a British journal, I failed to notice that the copy editor had everywhere altered the common noun universe into the proper noun Universe. When the article was published, I realized too late that this apparently harmless alteration had changed in a significant way my intended meaning. By no stretch of the imagination can a universe become the Universe.

Elsewhere¹ I have pointed out that current knowledge and usage assign different meanings to universe and Universe. The Universe means everything, including ourselves, and no doubt our descendants in the distant future will still be trying to understand the ultimate nature of reality. Nobody knows what is the Universe. Illogically, as a proper noun in common use, Universe denotes nothing more than a model of the Universe. When editors delete "universe", they should substitute not "Universe," but "model of the Universe".

The more modest and flexible word universe usually denotes "a model of the Universe". By using universe we avoid the meaningless Universe and the terminological rigmarole of model of the Universe. Every society has its universe or model of the Universe. We in the twentieth century have our physical universe, which is still a long way from being the Universe. We debate the rival merits of big-bang and steady-state universes, and under the rubric early universe discuss the virtues of the inflationary universe. (What a mess the last sentence becomes if Universe is substituted for universe!) Our descendants, though part of the same Universe, will live in different universes. In the history of cosmology we study the rise and fall of universes, or cosmic belief-systems, or models of the Universe, but not of Universes.

Indiscriminate use of Universe confuses the real thing with a model. As a result we forget that we have precious little knowledge of the true nature of the Universe. Like our forebears in past millennia, we tend to think that the end of the search for all knowledge looms in sight. Most people in the past, in the Middle Ages for example, were convinced that they had discovered the real thing. People with different models often finished up at the stake.

There exists only one Earth, and similarly only one Universe. But the difference is vast in more senses than one. With the first we know more or less what we are talking about, but with the second we have no idea. The curious words earth and Universe found in many journals and books look most odd. Logic dictates that we capitalize proper nouns such as Earth,

Sun, Solar System, Galaxy, Local Group, and leave it at that.

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¹ Harrison, E R *Cosmology The Science of the Universe* (Cambridge University Press, New York, 1981)

Imunovir

SIR — Replying to my letter¹ criticizing the UK campaign for the launching of isoprinosine (or Imunovir) as an effective anti-herpes treatment, Helen J. Wright made, on behalf of the laboratory commercializing the drug, numerous assertions and insinuations². I feel obliged to comment very briefly on some of them; the readers of *Nature* will have no difficulty in seeing most of the contradictions and the reasons for this defence.

To call W.H. Wickett's drugstore book³ an objective "independent text" on herpes does not correspond to reality. The author conducted, on behalf of the manufacturers (Newport Pharmaceuticals), a non-controlled clinical trial on herpes patients, and he reported his results in this book. If the name of the "test drug" is not mentioned until p.217, and this on the express request of the manufacturers, it is mentioned 12 times in the references between pages 218 and 225. The tantalized reader will have no difficulty in discovering the substance producing the wonderful results. But there is something more puzzling: Wickett apparently published his results only in this book and reported them at a couple of scientific meetings. Why do such extraordinary results, and those obtained by others using the same drug and cited by H. Wright, remain at the stage of meeting abstracts, which even a computer survey fails to pick up?

Bona fide readers who saw the various assertions concerning the drug are still wondering what the drug really does for herpes patients, whether it cures the "bouts"^{2,3} or diminishes to "nearly zero" the frequency of the attacks⁴. If this were the case, the number of patients should indeed decrease.

I am not surprised that articles such as those in the *Financial Times* and the *Sunday Times* promising a miraculous cure for diseases of great psychological impact — even if the titles of articles are written, as H. Wright suggests, by "irresponsible sub-editors" — are found "well balanced" by those whose main concern is the sale of the drug. I maintain that articles written for the lay public should be underplayed rather than exploit the spectacular. Readers of lay publications do not usually read journals such as *Nature*, where statements are made with extreme care, despite the fact that, or perhaps because, the readers

take everything with several pinches of salt.

This is the case of C. Wenz's article where the author cautions, because of the placebo effect, against drawing hasty conclusions in assessing the efficacy of Imunovir "until more clinical trials have been completed"⁴.

H.J. Wright has confirmed my belief that only those with vested interests would reject my criticisms and the idea of exercising self-censorship against aggressive publicity. I am certain that most will refrain from such practices and I also hope that the lay media will adopt a more restrictive policy in printing news related to health matters.

DIMITRI VIZA

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¹ Viza, D. *Nature* 313, 344 (1985)

² Wright, H J *Nature* 314, 126 (1985)

³ Wickett, W H Jr *Herpes: Cause and Control* (Pinnacle, New York, 1982)

⁴ Wenz, C *Nature* 311, 404 (1984)

Fuels from the farm

SIR — There seems to be an error in Anna Lubinska's article (*Nature* 4 April, p.395). It is stated that 8 million tonnes of cereal surpluses could produce 2 million tonnes of ethanol and that 1 hectare of land can produce 4 tonnes of ethanol. This would mean that 1 hectare of land produces 16 tonnes of cereal grain, but that is surely not the case. A good average yield through the countries of the European Communities is nearer half this figure. It should be borne in mind that the vagaries of the cereal intervention system ensure that it is the less efficient farmer who actually produces the surplus. These farmers are more likely to produce 4 tonnes per hectare of low quality cereals. The immense complications of agricultural production should be borne in mind when calculating potential hydrocarbon energy production by farms.

P.J. RYAN

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Nullius in verba

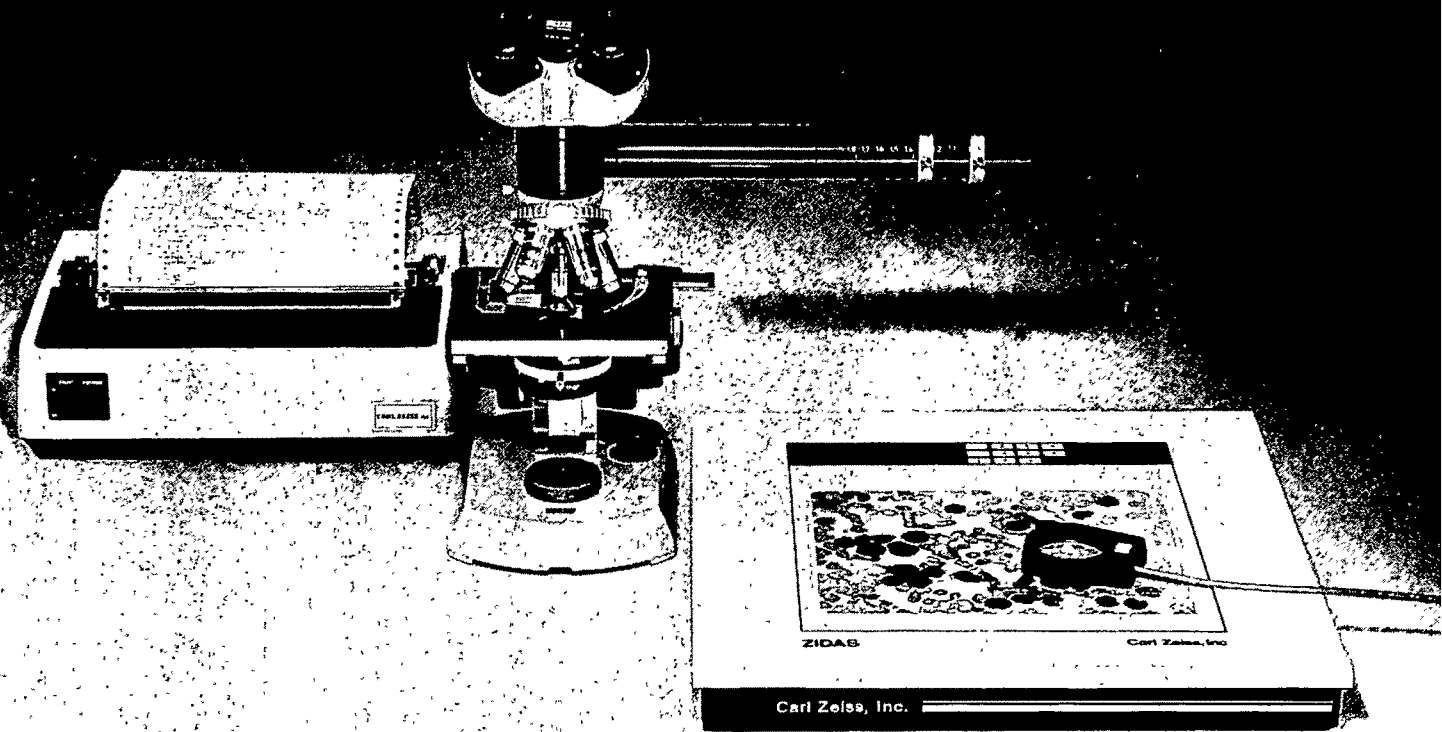
SIR — It was good to have the exact meaning of the Royal Society's *Nullius in verba* explained by the president (see *Nature* 23 May, p.272). In all fairness to young scientists, however, he should have urged caution in taking the society's motto as a rule to walk by. For those who reveal too early their realization, which will come soon enough if they are independent thinkers, that many authorities of the day are emperors with very threadbare clothes, the going is likely to be rough.

P. R. BELL

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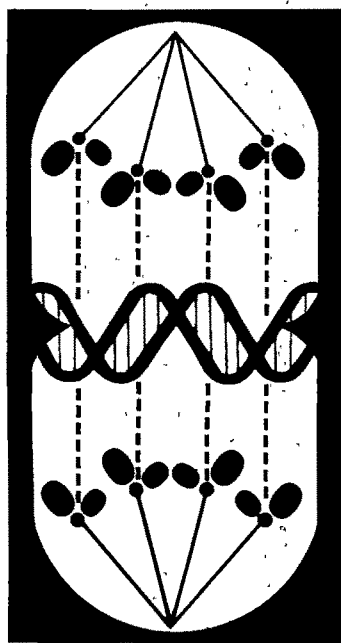


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systems

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Early development—
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CELLS

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receptors
B cells

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proteins
Yeast
development

Plant
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GENES

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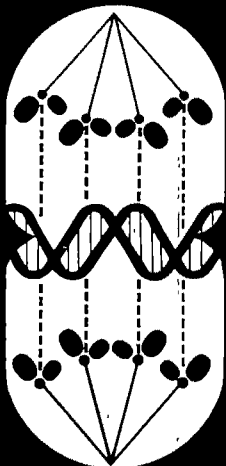
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Uniting mechanics and statistics

An adventurous scheme which seeks to incorporate thermodynamics into the quantum laws of motion may end arguments about the arrow of time — but only if it works.

THE logical relationship between the laws of mechanics and those of thermodynamics deserves more attention than it usually receives. Thermodynamics and statistical mechanics are ways of describing the behaviour of macroscopic systems made from components whose behaviour is determined by the laws of mechanics, classically those of Newton (as amended), but otherwise the equations of motion of quantum mechanics. Where the first law of thermodynamics is concerned, there is no difficulty. In both classical and quantum mechanics, total energy is a constant of the motion and is thus always conserved, at least in a closed system.

The difficulty arises chiefly with the second law of thermodynamics, and not only because there is such a variety of ways in which this principle can be defined. But now a group of three theoreticians has put forward an intriguing way in which the laws of quantum mechanics may be modified so as to incorporate the second law from what appears to be the outset (Beretta, C. P., Gyptopoulos, E. P. & Park, J. L., *Il Nuovo Cimento B* 87, 77–97; 1985). Whether the modification proposed is sufficient, only time will tell, but the objective seems well worth the trouble Beretta *et al.* have taken.

The difficulty is well illustrated by the way in which some kind of correspondence is established between the mechanical behaviour of a system and its thermodynamic properties. For more than a century, people have been brooding on the paradox that while the laws of classical (and, for that matter, quantum) mechanics are symmetrical with respect to time inversion, the second law selects from all possible trajectories of motion only those corresponding to a continual increase of the entropy. The arrow of time is conjured like a rabbit from a hat.

The definition of entropy in terms of the mechanical properties of the constituents of a system is similarly clouded. The classical model is Boltzmann's H -theorem (1872), which shows that the rate of change with time of a certain mathematical construct from the probability distribution of single particles in phase space will always be zero or negative. So Boltzmann argued, his quantity H is admirably suited to be the negative of what is known in thermodynamics as entropy. This is argument by analogy, but none the worse for that — if it works.

Since Boltzmann's time, there has

accumulated a rich literature on the implied paradox of the conflict between the irreversibility of macroscopic processes and the reversibility (in time) of the laws of mechanics and thus of microscopic processes. Indeed, the argument was begun by Loschmidt in 1876, but now even elementary text-books of thermodynamics reckon to give some kind of account of it.

The standard explanation is that the apparent paradox is not a paradox at all, but a confusion about timescales. Any measure of entropy, that derived from Boltzmann's H or otherwise, will fluctuate (and so decrease as well as increase on a short timescale), which is not inconsistent with the notion that the average value of the entropy should increase steadily over long periods of time (or remain unchanged when the system is in equilibrium).

Much the same is said of the recurrence paradox, based on the observation due to Poincaré that the point in phase space (momentum as well as position) representing the state of a classical system will return to more or less the same place after a sufficient length of time. On the face of things, that means that non-equilibrium states of a system will repeatedly recur. The standard resolution of that paradox is the observation that, for any realistic system, the interval of time between recurrences will be huge, much greater than, say, the age of the Universe. Again there is nothing wrong with these arguments, but they are far from being rigorous.

So why not take the bull by the horns, and build irreversibility into the laws of mechanics? That is the point from which Beretta *et al.* start. Properly, they acknowledge that they are not the first to tread this path. They work with quantum statistical mechanics, where the formalism is easier. They start from the equation of motion for the operator representing the state of a physical system, say m , which is, in operator language, $dm/dt = -i/\hbar [H, m]$, where t is time, H the Hamiltonian operator of the system and i and $\hbar$ the square root of minus one and Planck's constant (divided by 2π) respectively. The quantity in square brackets is the commutator of its two components, $mH - Hm$.

The natural way to proceed is to assume that this equation is modified in such a way that the right-hand side is some other function of the state operator m than in the standard form. The objective is to find a form of the function which is compatible

both with what is known of the evolution of thermodynamic systems and, perhaps more important, the dynamics of real microscopic systems. Beretta *et al.* have convinced themselves that the function they are seeking cannot be a linear function of m . What they propose is the addition to the right-hand side of the quantum equation of motion of a particular function of m which, by including both the square root and the logarithm of the state operator of the system, is non-linear enough to satisfy anybody's taste.

Almost magically, the system has some of the obviously necessary properties. For example, for a system in a pure quantum state, say that represented by a solution of Schrödinger's equation, the extra terms vanish and the simple form of the equation of motion applies. Similarly, constants of the motion in the new system are also constants of the motion determined by the simpler equation of motion.

What can be said about the entropy? In reality, the state operator m is the equivalent of what is called the density matrix in quantum statistical mechanics, which is why Beretta *et al.* define entropy in terms of the operator m log m , where the logarithm is the natural logarithm of the operator m . Specifically, the entropy of the negative of the trace of this operator multiplied by Boltzmann's constant; the authors are able to show that it increases (or does not increase) in the course of time.

So is this a demonstration that the laws of mechanics and of thermodynamics can indeed be combined? Not quite. For one thing, there are various mathematical problems that make some of the steps in the argument conjectural. Worse still, some of the operator functions in the formalism are sometimes undefined. But the system does have the merit of hanging together — the paper now published extends to composite systems the treatment of one-component systems published a year ago.

None of this implies that the arguments about the reconciliation between microscopic reversibility and macroscopic irreversibility will now be stilled. Indeed, while for as long as the present justification of the basis of statistical mechanics holds water, there will be many who say that what Beretta *et al.* have done is strictly unnecessary. But this is a field in which the proof of the pudding is in the eating.

John Maddox

Acquired immunodeficiency syndrome

Laying the groundwork for neutralizing AIDS-linked virus

from Jerome E. Groopman

WITH what success does the human immune system produce antibodies that neutralize the human T-lymphotropic virus type III (HTLV-III) — the virus that is aetiologically linked to the acquired immunodeficiency syndrome (AIDS)? And can the neutralizing antibodies be exploited for prophylaxis or therapy of AIDS? Two papers elsewhere in this issue^{1,2} report that such antibodies are present in some sera both from AIDS patients and from people who have been exposed to the virus but are asymptomatic. The relatively low titres of neutralizing antibodies in HTLV-III-infected persons should not dissuade investigators from pursuing their clinical relevance; the feline leukaemia virus often elicits low titres of neutralizing antibodies *in vivo*,³ yet vaccines of some effectiveness, which exploit at least in part this antibody response, have recently been developed.

Several studies that would help to define the significance of HTLV-III neutralizing antibodies *in vivo* immediately suggest themselves. Comparison of the titres and types of these antibodies in asymptomatic and AIDS patients can readily be accomplished. Particularly interesting would be longitudinal studies to examine whether the neutralizing antibodies persist in the sera of those who remain relatively healthy despite exposure to the virus but diminish in those to progress to AIDS. Of course, the clinical situation may not be so simple. Like visna virus, to which it bears some similarity, HTLV-III may alter its surface antigenic determinants over time and 'escape' from the protective effects of neutralizing antibodies^{4,5}. Serial culture of HTLV-III with testing of neutralizing antibodies on the host's own isolates is one approach to determine if *in vivo* there are selection pressures that favour the emergence of antigenically altered virus. Initial reports of apparently healthy adults in Central Africa who are both antibody positive and virus positive for HTLV-III should make this population particularly valuable in addressing the clinical role of neutralizing antibody⁶. Because antigenic determinants on the gp120 envelope protein of the gp41 transmembrane protein of the virus^{7,8} are likely to be the primary sites of interaction with the putative cellular receptor for HTLV-III^{10,11}, it is to be predicted that neutralizing antibodies are directed against gp120 and/or gp41 determinants. The wide antigenic variability in envelope-related antigens from different HTLV-III isolates¹² makes testing the ability of geographically-distant sera to neutralize geo-

graphically-distant isolates a valuable means of identifying conserved epitopes for neutralizing antibodies.

Recently, a retrovirus that cross reacts in serological tests with HTLV-III has been isolated from African Green monkeys¹³. Studies on the neutralization of HTLV-III by sera from these monkeys and of the ability of human HTLV-III neutralizing antibodies to recognize the surface determinants of the monkey virus may also help to localize highly conserved epitopes that would interact with cellular receptors for the viruses.

HTLV-III, LAV and ARV — all clearly variants of one type of human retrovirus — have been cloned and sequenced, thereby providing ready access to recombinant (made by recombinant DNA technology) viral peptides¹⁴⁻¹⁷. Peptides of the envelope protein can be used *in vitro* to absorb out the neutralizing activity and localize the epitopes that elicit antibodies. It is of interest to determine whether some neutralizing antibodies might react with the putative gene products (termed sor, P', Q and 3'ORF, E', F) that may have functional roles, particularly in the activation of cellular genes (transactivation). The availability of these products will allow the reverse test: do polyclonal or monoclonal antibodies raised in animals against any of these gene products have *in vitro* neutralizing capacity? To obtain the equivalent human antibodies, it may be possible to use B-cells from people with neutralizing antibodies.

Ultimately, *in vitro* studies will be needed to determine the clinical signifi-

cance of neutralizing antibodies for either prophylaxis or therapy of HTLV-III infection. Initial tests of prophylaxis with hyperimmune globulin and of therapy with pools of high-titre neutralizing antibodies can be undertaken in primates which are susceptible to infection with HTLV-III. Simple vaccination with native or recombinant peptides that elicit neutralizing antibodies may not be sufficient to protect primates against challenge with HTLV-III, but such an approach can readily be examined. The role of the cellular immune response (in addition to the antibody response) in preventing or suppressing human retroviral infection is still unaddressed. Inoculation of primates with forms of HTLV-III that are defective in functional genes but still display structural antigens, or with recombinant constructions of HTLV-III with other viruses, such as vaccinia, should permit the study of both cellular immune and antibody responses.

The HTLV-III research community will now coordinate its clinical and scientific forces to build on the new observation of neutralizing antibodies in the hope of constructing strategies to outwit this threatening human retrovirus. □

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Lasers

Dynamic holograms from crystals

from Malcolm Gower

WHEN certain crystals are exposed to spatially non-uniform light fields, light-induced refractive index changes occur, caused by the migration and retrapping of charges. Known as the photorefractive effect, the phenomenon was first reported nearly twenty years ago as the mechanism responsible for the self-focussing of laser light in crystals of LiNbO₃ and LiTaO₃, and has since been observed for many more crystals, including CdS, InP and GaAs. It was soon recognized that photorefractive crystals used as three dimensional media for holographic recording (volume holograms) could contain extremely high densities of information,

up to 10¹² bits per cm³, yet retain exposure sensitivities comparable to those of the best photographic emulsions (at least 100 μJ per cm²). But, unlike conventional media, photorefractive crystals can be used for dynamic or 'real-time' holography in which the writing and reading processes occur virtually continuously and simultaneously. Recently there have been some remarkable discoveries in the field that may lead to a host of applications in real-time optical data processing — from optical image amplifiers, enhancers and pattern recognizers to optical switches and memories¹.

Unlike the relatively slow serial proces-

sing that occurs in conventional electronic devices, dynamic holograms allow rapid, parallel-bit processing of information. Because the image wave is a complex or phase conjugate of the subject wave, real-time holography is closely analogous to the nonlinear optical method of degenerate four-wave mixing used in producing phase conjugate (wavefront reversing) mirrors (see Fig. 1a; ref. 2). Thus these crystals can also be used as phase conjugate mirrors to correct distortions on optical wavefronts and to project complex images.

The hologram in photorefractive crystals results from the migrating charges, which may be either positive, as in BaTiO_3 or negative, as in $\text{Bi}_{12}\text{SiO}_{20}$ and $\text{Bi}_{12}\text{GeO}_{20}$. The charges are generated by photoexcitation of electrons or holes from impurity or dopant levels in the band gap of the crystal. The interference pattern set up between the subject and reference waves during recording causes the mobile charges to diffuse or drift (photoconduct) and become retrapped in the darker regions of the interference pattern, leaving behind trapped ionized centres (Fig. 1b). This spatial separation of charge creates local piezo-electrically induced stresses and strains caused by electric fields whose gradients (by Poisson's equation) are a maximum in regions of maximum space-charge density, ρ_{sc} , so that the space-charge field E_{sc} is often phase shifted from the original interference pattern (Fig. 1b). The space-charge field then produces local variations of the refractive index,

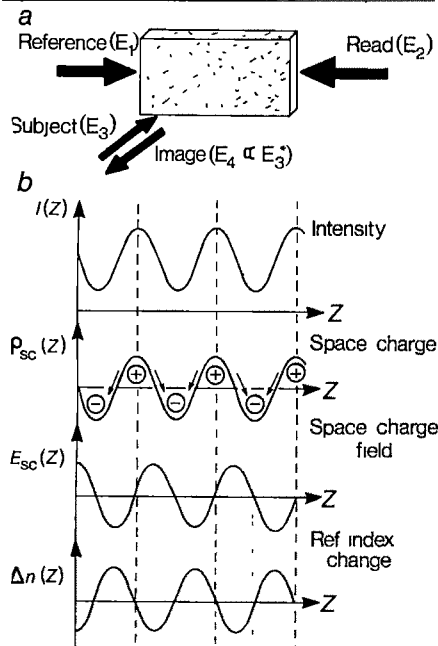


Fig. 1 a, Dynamic holography: arrangement for producing real-time holograms and degenerate four-wave mixing phase-conjugate mirrors. **b**, Photorefraction: an interference pattern causes charges produced in bright regions to migrate into and become trapped in dark regions, creating a phase-shifted space charge field, which causes variations in refractive index. The case is shown for negative charge carriers Z is the space coordinate; for other variables see text.

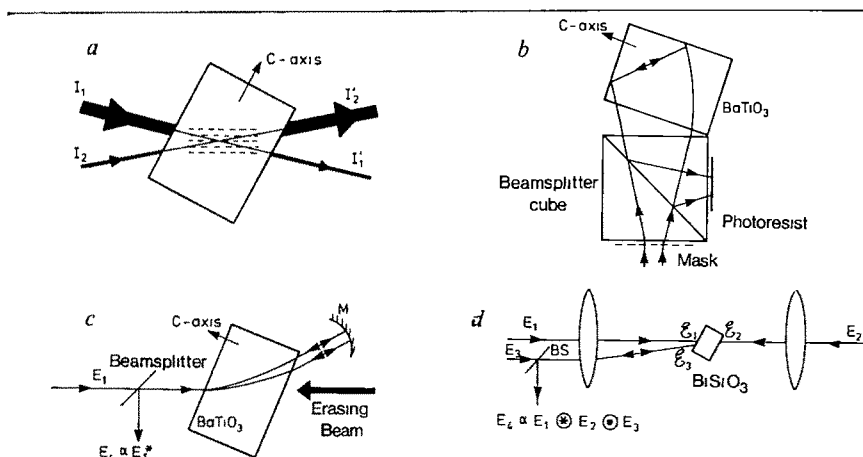


Fig. 2 a, Two-beam coupling in phase-shifted holograms. **b**, High-resolution image projector using a self-pumped BaTiO_3 crystal. **c**, Self-pumped, optically bistable phase-conjugate reflector. **d**, Spatial convolution $\otimes$ and correlation $\odot$. ϵ_1 , ϵ_2 , ϵ_3 are Fourier transforms of the writing and reading beams E_1 , E_2 , E_3 .

Δn , throughout the medium as a result of the linear electro-optic Pockels effect. The hologram may be permanently 'fixed', by applying a biasing voltage across the crystal during recording, or can be erased by uniform illumination of the crystal.

The maximum modulation of both the interference pattern and the induced space-charge field occurs when the recording beams have the same intensity, irrespective of their absolute values. Thus, in contrast to most other mechanisms used to produce phase-conjugate mirrors, the normalized image-wave light intensity (phase-conjugate reflectivity) produced by the reading process is insensitive to the absolute intensity of the recording beam. In BaTiO_3 , for example, which has an extremely large electro-optic effect, an image-intensity gain of 100 has been observed using low-powered continuous lasers³. As the crystal behaves like a phase-conjugate mirror with gain, oscillation could be produced between it and a relatively poor, non-aligned reflector, such as a kitchen spatula. When the spatula is moved, oscillation reappears at its new position, demonstrating that the mirror can automatically track its whereabouts.

If the intensity of the recording reference beam is about half that of the subject beam, then during reading maximum reflectivity for transmitted light occurs near the edges of patterns placed in the subject beam, where the intensities of the two recording beams are equal. Such preferential enhancement of the edges of the image is often beneficial when trying to recognize complicated patterns. Because the rates for holographic recording and erasure depend on the photoconductivity of the crystal, which in turn is proportional to the intensity of illumination, using high light intensities for recording and erasure increases these rates. Thus, in BaTiO_3 , for example, a hologram that takes a few seconds to record using a low-powered He-Ne laser requires less than 10 nano-

seconds when an intense (20 MW per cm^2) pulsed laser is used. On the other hand, provided the crystal is kept dark, the recorded hologram can be stored for long periods, from a few hours to over 10 years depending on the crystal species. Storage is limited only by the dark conductivity of the crystal, which eventually redistributes the charge carriers.

If a weak and a strong beam intersect in a crystal (Fig. 2a), most of the energy in the strong beam can be transferred to the transmitted weak beam (since the Bragg reflection of one wave from the hologram is phase shifted by $\pm \pi/2$ from the transmitted wave of the other, the two waves will interfere constructively or destructively if the hologram is additionally shifted by $\pi/2$). Because the volume hologram written in the crystal is often phase shifted from the original optical interference pattern (Fig. 1b), the two writing beams can exchange energy by their self-diffraction from the hologram. This 'two-beam coupling' effect, although complicating the understanding of many observations in photorefractive dynamic holography, leads to some remarkable effects. For example, in BaTiO_3 , where diffusion processes alone cause charge migration, the hologram phase shift is $\pi/2$ and intensity gains of 600 have been observed for the transmitted weak beam. A two-dimensional laser-beam steering device, using two-beam coupling for the potential random accessing of optical memories, has recently been constructed⁴.

Furthermore, two-beam coupling between a single laser beam and scattered light propagating in the appropriate direction in a BaTiO_3 crystal can lead to self-defocussing or 'fanning' of the beam (see cover picture). If there is total internal reflection of the fanned beam from the far corner of the crystal, phase-conjugate reflections can be produced with a reflectivity of up to 30 per cent by using just one input beam to the crystal⁵. We have recently used such a self-pumped phase-conjugate mirror, with the arrangement

shown in Fig. 2b, to project speckle-free images with submicron resolution. Such a system may be useful in photolithography, as used in the production of silicon chips, for projecting high-resolution images over a large area.

Because two-beam coupling effects are inherent in phase-shifted volume holograms, a self-starting, self-pumped phase-conjugate mirror can be produced by feeding back the transmitted fanned beam to the crystal (Fig. 2c; ref. 6). When the intensity of a separate erasure beam is continuously varied, the reflectivity of this device can have multiple values and exhibit hysteresis effects. This optical bistable behaviour may prove useful for high speed switching and memory elements in future optical computers^{7,8}.

By using lenses to focus and take Fourier transforms of the writing and reading beams, dynamic holography has been used to perform the real time operations of convolution and correlation in crystals of $\text{Bi}_{12}\text{SiO}_{20}$ (Fig. 2d; ref. 9). These operations are extremely useful in complex image pattern recognition, and for image deblurring and enhancement.

Furthermore, by using relatively large intensities to illuminate the subject, dynamic holography in photorefractive media can be used to create a beam that

is the mathematical division of two images¹⁰. By making use of the relatively slow recording and erasure times of these media, it is also possible to perform the real-time subtraction and differentiation of images. Compared to the numerical computational techniques now in use, optical images can in principle be manipulated much more rapidly by direct optical methods.

With their many potential applications in dynamic holography, from optical memory storage and switching to image projection and synthesis, photorefractive media may well produce a revolution no less dramatic than that caused by the discovery of photographic materials over a century ago. □

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Immunology

Immune response restored by gene therapy in mice

from Michael Steinmetz

Two classes of cell surface molecules encoded in the major histocompatibility complex (MHC) genes are known to guide T cells of the immune system in their recognition of foreign antigens on cell surfaces. In general, cytotoxic T cells recognize foreign antigens in association with class I molecules, whereas helper T cells co-recognize antigen and class II molecules. The precise molecular interactions that lead to the detection of foreign antigens in the context of MHC molecules (termed MHC restriction) are not known and the role that the MHC antigens play in the development and functions of antigen recognition by T cells is poorly understood. But it is beginning to look as if transgenic mice will provide some answers.

Over the past five years MHC genes, including those encoding for the T-cell restriction elements, have been cloned and sequenced both from mouse and man (see refs 1 and 2). By transfecting genetically distinct cells growing in culture with class I and II genes it has been shown that the molecules they encode function appropriately as T-cell restriction elements³. Currently, these types of gene transfections are being combined with exon-shuffling experiments to identify

which protein domains in class I and class II molecules are recognized by cytotoxic and helper T cells, respectively. One hopes that these experiments, perhaps in conjunction with X-ray crystallography, will eventually define the amino-acid residues that make contact with T-cell receptor molecules and those that may make contact with foreign antigen.

Gene transfer into somatic cells, however, is of limited use. Questions concerning the expression and regulation of genes during development and in differentiated cells, and their influence on the developing individual, are best approached through germline DNA by, in particular, micro-injecting genes into the pronuclei of fertilized eggs (to produce transgenic animals) or using retroviral vectors to transmit genes into early mammalian embryos (or bone marrow cells)⁴. With respect to the immune system there are a series of questions concerning the generation of the diverse repertoire of B-cell and T-cell antigen receptors that can be studied in transgenic animals. In the past two years, several investigators have introduced functional genes for antibody molecules into the mouse germ line to study the tissue and cell specificity of

regulation, and the mechanism of allelic exclusion, of immunoglobulin genes in B cells^{5,7}. Now, transgenic mice have been produced with cloned MHC genes to find out whether the introduced gene can be expressed in a functional way.

Frels *et al.*⁸ have introduced a porcine MHC class I gene into mouse germline DNA and have shown that the foreign gene is accurately expressed in the transgenic mouse. All cells examined display the porcine class I molecule on the surface, and skin transplanted from the transgenic mouse to a normal mouse of the same strain is rapidly rejected. It is known that genetic differences in class I molecules will induce rapid graft rejections — hence they are also termed transplantation antigens. The findings of Frels *et al.* indicate that the porcine class I molecule is recognized by mouse T cells as a transplantation antigen and that the immune system of the transgenic mouse has become tolerant to the porcine molecule. Whether the porcine class I molecule can also function as a restriction element in T-cell-mediated antigen recognition in the transgenic mouse still remains to be investigated.

A related question, namely whether class II antigens encoded by genes introduced into transgenic mice can function as T-cell restriction elements, has now been answered in elegant experiments carried out by three independent groups, two of which describe their results in this issue^{9,10} whereas the third has a paper submitted (L. A. Pinkert *et al.* personal communication). Normally, mice have two different class II molecules, A and E, both of which are composed of distant α - and β -chains. For their experiments, all three groups have made use of strains of mouse that have a 5'-deleted E_α gene and can no longer synthesize the E_α molecule. As a result, their E_β molecules, which are produced as normal, remain in the cytoplasm of the cell rather than forming a heterodimer with E_α on the cell surface. The E_α -deficiency of these mice has previously been correlated with their inability to generate an immune response against certain antigens, for instance to the synthetic copolymer composed of glutamic acid, lysine and phenylalanine (GLPhe).

To perform gene replacement therapy the different investigators used previously cloned intact E_α genes derived from two different mouse strains which contain almost identical E_α polypeptide chains. The intact E_α genes were micro-injected into fertilized eggs of the immune-deficient mice, leading to the birth of a few transgenic mice that contained several copies of the introduced E_α gene. These genes are inherited in a mendelian fashion by the offspring of the transgenic mice, indicating that they are integrated into one autosomal chromosome, and that they are transcribed into messenger RNA molecules of the right size and, more importantly, only in those cells that normally

express class II genes. In contrast to the immune-deficient mice used as recipients, the transgenic mice display an E_a/E_b complex on the appropriate cells indicating that the introduced E_a gene encodes an E_a polypeptide that associates with the host-encoded E_b molecule. Furthermore, Le Meur *et al.*⁹ and Pinkert *et al.* find that the expression of the transgenic E_a gene can be correctly induced in macrophages by γ -interferon. In contrast, Yamamura *et al.*¹⁰ find that the introduced E_a gene and the endogenous E_b gene are constitutively expressed in macrophages. The reasons for the discrepancy are not clear. Taken together, these results show that accurate regulation of the E_a gene can be achieved (despite presumably random DNA integration) with cloned DNA fragments that contain, in addition to the E_a gene, as little as 2 and 1.4 kilobases of 5'- and 3'-flanking sequence, respectively.⁹

The transgenic mice were also shown to have a restored immune response against the antigen GLPhe. Unlike the mice that did not contain an intact E_a gene they could specifically recognize the antigen as measured by T-cell proliferation and antibody production^{9,10}. This shows that reconstitution of the proper class II restriction

element, which is needed to mount an immune response against the particular antigen tested, has been achieved.

These experiments suggest that it will be possible to endow mice with new MHC restriction elements that can function properly in the generation of an immune response. Until now, manipulation of MHC genes in living animals has required the breeding of F_1 hybrid mice, genetic recombination experiments, the time-consuming isolation of mutant mice and the complicated construction of chimaeric mice. Now, new, additional or *in vitro*-modified MHC genes can be introduced into transgenic mice where their effects can be studied *in vivo*. □

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Photoelectrochemistry

Quantization effects in superlattice photoelectrodes

from Mary Archer

ARTHUR NOZIK'S strained-layer superlattice photoelectrode, reported in this issue (*Nature* **316**, 51; 1985), is a neat example of technology transfer. Superlattice or multiple-quantum well structures are two-dimensional solids in which thin layers of two different materials A and B, usually semiconductors, are alternated (ABAB) or sandwiched (BAB) one with the other. Electrons localized in ultra-thin (roughly 3–25 nm) semiconducting layers produce unique solid-state physics in superlattice structures, which is now being applied to the possible production of better transistors and solid-state lasers. Nozik has used this unique physics to produce a photoelectrode that could be the prototype of a new generation of photoelectrochemical devices for the conversion of solar energy to electrical power.

What is special about the electrons trapped in a thin-layer region? As every first-year student of quantum mechanics knows, the translational energy levels E available to a particle of mass m in a one-dimensional box of length a are given by $E = n^2 h^2 / 8ma^2$, where h is the Planck constant and n (>1) is the quantum number. An electron localized in a layer of sufficiently small thickness a behaves differently from an electron free to move over larger distances because its energy levels

are coarsely spaced rather than quasi-continuous. In a superlattice structure, the energy levels in the two dimensions parallel to the layers are normal but those in the short dimension through the layers are coarsely spaced. Attractive properties of superlattice devices can be tailor-made in that the electronic and spectral properties are tailored by the thickness of the layers.

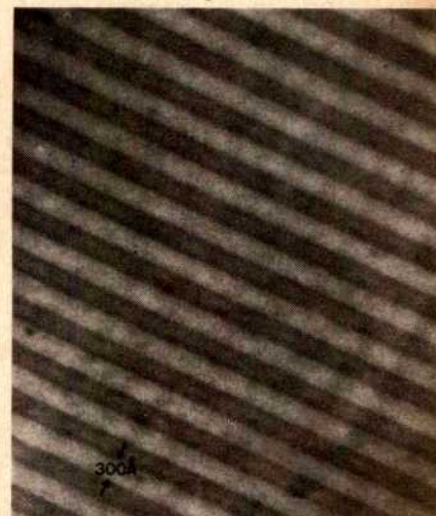
All this has been known in principle for decades, but the technology of fabrication of layered structures of good quality, essentially free from the damaging effects of surface states at the A/B interfaces, is much more recent. The production of GaAs/GaAlAs layered structures was pioneered by Bell Laboratories in the 1970s. Strain-free lattice-matched layers of these two materials can readily be produced because their lattice parameters are very similar. However, the number of pairs of materials with good lattice match is very limited. Lattice mismatch at the A/B interface cannot be tolerated because it produces a high density of surface states and superlattice structure of very poor quality. The exploitation of materials with dissimilar lattice parameters has now become possible with the discovery that suitably thin A layers can be epitaxially laid down on B to produce a strained struc-

ture, in which the lattice parameter of A is greater than in the bulk material and is matched to that of B. Fabrication, by molecular-beam epitaxy or metal-organic chemical vapour deposition of strained-layer superlattice structures with acceptably low surface-state density has been developed by the Sandia Corporation, Albuquerque in the past couple of years.

At present there seem to be three main possible applications of these quantum-well structures. The first is the production of a new generation of solid-state lasers. Existing solid-state lasers contain p - n homojunctions within a single semiconductor. Now GaAs/GaAlAs and other A/B heterojunction solid-state lasers of superior properties are being developed. These heterojunction lasers have the potential advantage that the lasing wavelength can be tailored by tailoring the thickness of the doped GaAs layers.

The second application is to the production of better field-effect transistors. Again, GaAs and GaAlAs are most often used. Doping of the active GaAs layer is achieved in a novel way by incorporating the Si dopant in the GaAlAs top layer. Electrons from the Si dopant atoms pass to the GaAs layer, rendering this layer electronically n -type but leaving it free of dopant ions. Thus, there is virtually no impurity scattering of electrons in the GaAs layer and its resistivity, which in a conventionally doped material at low and moderate temperatures is dominated by impurity scattering, is low. Consequently the losses in the transistor are less so that less electrical driving power and heat sinking are required.

The third possible application is to heterojunction photovoltaic detectors that could be fabricated on a chip and would be particularly suitable for use in combination with optical fibres. These have maximum transmission at about 0.8 eV so Si-based photovoltaic detectors,



Superlattice structure consisting of alternating layers of GaAs_{0.45}P_{0.55} (dark layers) and GaAs_{0.75}P_{0.25} (light layers) that are 275 and 300 Å thick, respectively. The light layers form quantum wells and the dark layers potential barriers (photo courtesy of K. Jones).

with a band gap of 1.1 eV, cannot be used.

Reverting to photoelectrochemistry, quantum size effects produced by very small (<10 nm) semiconductor particles have already been reported. Nozik now describes the first quantum size effects produced in a photoelectrochemical system using a superlattice structure (see figure). The onset of photocurrent from a conventional GaAs thick-layer photoelectrode occurs at the band edge (1.42 eV) of the material, rising monotonically to a saturation value at lower wavelengths. The photocurrent action spectrum (fig. 3 of Nozik's article) of the GaAs/GaAsP strained-layer superlattice photoelectrode is quite different, showing the multiple peaks that should result from transitions between the discrete energy levels in the quantum wells.

Is this photoelectrode useful or merely interesting? It is hard to answer this question as yet. Its present quantum efficiency is poorer than that of a conventional bulk GaAs photoelectrode. On the other hand, the ability of superlattices to absorb photons of discrete energies provides a means whereby higher solar-energy con-

version efficiencies might be achieved. Conventional photoelectrodes degrade the energy of absorbed photons of energy $E > E_g$ to the band-edge energy E_g , thereby dissipating a significant fraction of solar energy as heat. This could be avoided in a superlattice photoelectrode because carrier relaxation to the semiconductor band edges should be inhibited by the presence of discrete energy levels with relatively large separation.

Two-dimensional solids are also excellent vehicles for basic physics. Their fabrication by molecular-beam epitaxy and chemical-vapour deposition is being investigated in several laboratories. "A lot of new physics will come out of two-dimensional solids" says Dr Michael Pepper, the leader of the Cavendish Laboratory's team. "For example, optical logic devices, in which thin layers are optically switched between a non-absorbing and an absorbing state, become an attractive possibility". □

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Muscle structure

First sight of crossbridge crystals

from Roger Craig

TO DESCRIBE the precise atomic interactions responsible for generating the force that drives muscle contraction is the dream of muscle biologists. X-ray crystallography of contractile proteins should provide the answers but the purified proteins tend to form polymers, similar to those in muscle filaments, rather than the necessary crystals. Nevertheless, the structures of three components of the thin filaments — tropomyosin, troponin-C and actin — have all recently been resolved at atomic or near-atomic resolution (see ref. 1). And now, I. Rayment and D. M. Winkelmann have obtained and begun to analyse crystals of the head of myosin, the pivotal protein in contraction^{2,3}.

The head or subfragment-1 (S1) of the myosin molecule is the energy-transducing component of the contractile machinery and constitutes the cross-bridges, the cyclic interaction of which is responsible for the sliding of the actin filaments past the myosin filaments during force generation. There are two heads attached to one long tail in each myosin molecule; the tail is an alpha-helical coiled-coil which aggregates with other tails to form the backbone of the thick filament, whereas the heads are globular units, each comprising a heavy chain and two light chains, which together contain the sites for ATP hydrolysis and actin binding. The heads can be cleaved from the tail by proteolysis.

Myosin itself has never been crystal-

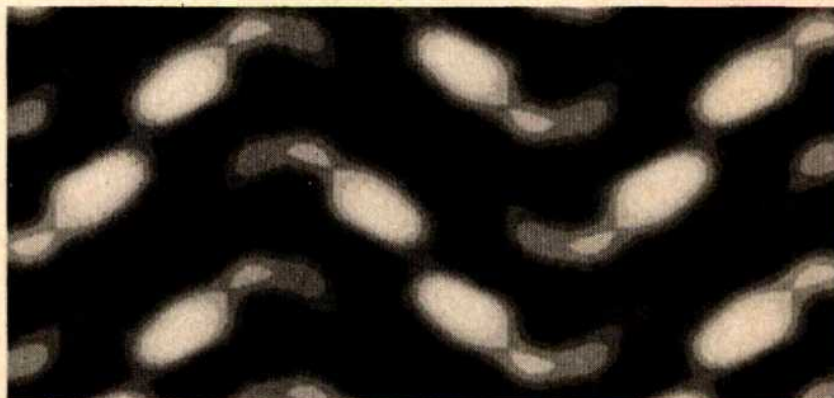
lized but much has been learnt from analysis of the purified heads and tail. Two-dimensional crystals (paracrystals) of the tail and its amino-acid sequence reveal repeating periodicities in the myosin filament backbone⁴. S1 is found to be an elongated (200 Å), curved, pear-shaped entity in the electron microscope (for example refs 5,6), although solution X-ray scattering⁷ suggests a somewhat different appearance.

But the crystallization of S1 has consistently eluded muscle researchers, leading to an assumption in some circles that the molecule could not be crystallized. Therefore, the two papers by Rayment and Winkelmann^{2,3} represent an unexpected breakthrough. Crystals of S1 diffracting to

at least 4.5 Å resolution have been grown by careful attention to the source of myosin and method of proteolysis. The relatively pure fast-twitch chicken pectoralis (breast) muscle was used as starting material, rather than the mixed-fibre muscles that are used in the more conventional rabbit myosin preparation. Hence, the myosin isozyme population was presumably more uniform than usual. Papain, in the presence of divalent cations, was used to digest the myosin instead of the more commonly used chymotrypsin. In this way S1 retains both of its light chains, known from other studies to be important in stabilizing the 'neck' region of the myosin head (chymotrypsin causes loss of one of the light chains).

It will no doubt be several years before the high-resolution structure of S1 is available. Meanwhile, in a departure from normal protocol, Winkelmann *et al.*² are studying the crystals by electron microscopy and resolving long-standing uncertainties⁴⁻⁷ about the gross structure of S1. From electron micrographs of fixed and embedded crystals sectioned in selected crystallographic planes, the authors have produced low resolution (about 30 Å) images of S1 in three different projections. Significant is the fact that optical diffraction patterns of the micrographs are similar to the X-ray patterns of the native hydrated crystals showing, reassuringly, that the micrographs present a reasonably faithful image of native S1 at 30 Å resolution. The most informative view of the crystal is that looking down the *a* axis (see figure). In this projection the long axis of the S1 molecule lies approximately in the plane of the section, and S1 molecules at different depths in the section are virtually superimposed on one another. Thus, each stain-excluding tadpole-shaped structure is effectively a projection of a single S1 molecule viewed through its thinnest dimension.

The overall shape and size of S1 can be deduced from these sections. The molecule is curved and its contour length is at least 160 Å (probably longer), providing direct and strong support for the shape and length seen in electron micrographs of shadowed myosin⁵ and in reconstructions



Filtered image of an electron micrograph of a S1 crystal, looking down the *a* axis. The 'negative' contrast of this specimen (protein, white; solvent regions, black) is a feature of the tannic acid/glutaraldehyde procedure used to fix the crystals (courtesy of D. A. Winkelmann).

of actin 'decorated' with S1 containing both light chains⁶. It is about 60 Å at its widest, tapering distally to a less dense neck region where the light chains are located and where attachment to the tail would occur^{6,8}. This length implies that shorter estimates, based on solution-scattering data⁷, are incorrect: it seems likely that these data do not readily pick up the presence of the thin part of the myosin head.

These electron microscope studies of the crystal represent only the beginning. The authors are extending their work to a full three-dimensional reconstruction of S1 based on tilt views of the crystals. As heavy-metal derivatives and higher resolution X-ray data are obtained, the three-dimensional courses followed by the heavy and light chains should become visible. Hopefully, the structural mechanism of ATP hydrolysis will be clarified. But the culmination should be when the three-dimensional structure of S1 is brought together with that of actin: then it may be possible to decipher the nature of the

activation of the myosin ATPase by actin and possibly even to deduce the kind of structural change that occurs when these molecules interact to produce force. Meanwhile these preliminary studies both whet the appetite and strongly support the gross picture of S1 suggested by electron microscopy. They also reassure electron microscopists (and their critics) that molecular structure can be well preserved even when proteins are stained, dehydrated and observed with an electron beam under high vacuum. □

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Crystallography

Some answers but more questions

from A. L. Mackay and P. Kramer

THERE has been an exponential explosion of activity among crystallographers following the report by D. Shechtman *et al.*¹ of a splat-cooled Al/Mn alloy showing, over extended regions, electron diffraction patterns of full three-dimensional icosahedral point symmetry. Some 25 follow-up papers, mostly preprints, are in circulation and several high-resolution micrographs of this material have been published. It remains to be seen whether the structure implies a conceptual revolution, a change of paradigm or just a shock to our complacency.

On page 50 of this issue, L. A. Bursill and Peng² publish a micrograph in which the spots fit very closely to the points in the 2-dimensional Penrose quasi-lattice (which is a section of the 3-dimensional icosahedral pattern). One characteristic feature is the arrangement of spots in a regular decagon, of diameter 14.5 Å, with an isosceles (non-equilateral) arrangement of three points inside. We might contrast this with the micrograph of the Sendai group³, where the triangles inside the rings are not resolved but which shows order over a larger area and where the long-period modulations show up clearly.

For the interpretation both of diffraction patterns and of micrographs, the problem of double diffraction is crucial⁴, as can be seen by considering five sinusoidal density waves at 72° to each other in the plane. The transform is a ring of 10 spots. If the diffracted waves are scattered again in the same specimen, the resulting diffraction pattern, the convolution of the

ring of 10 spots with itself, accounts for almost all of the observed diffraction spots (and repeated diffraction would account for the remainder). Thus, both diffraction patterns and micrographs tend to be self-similar, with ratios of the golden number, and it is difficult to decide which is the primary level.

Nevertheless, P. A. Bancel and others⁵ have obtained powder X-ray diffraction patterns using synchrotron radiation and claim to have eliminated multiple diffraction effects. Their powder pattern is indexed with six integer indices with respect to six axes (parallel to the 5-fold axes of the icosahedron). Given the earlier demonstration⁶ of how to handle standard crystallographic calculations with redundant axes such as these, the planes corresponding to the [10000] reciprocal-lattice vector turn out to have the spacing of 2.17 Å. The intercepts of this plane on the icosahedral axes, corresponding to the edges of the Penrose tiles, are $\sqrt{5}$ times as great, namely 4.85 Å, which fits reasonably with a 14.5 Å diameter for rhombic triacontahedra and would give about 5–6 atoms per acute rhombohedron. Portier and others report an edge-length of 4.60 Å for the proposed Penrose rhombohedra.

The relative stability of quasi-lattice and of normal crystalline phases has been investigated both experimentally and theoretically. G. Parthasarathy and the group in Bangalore report⁷ that high pressure (5–9 GPa, varying with composition) irreversibly converts the icosahedral phase to the crystalline, indicating that the

former is metastable. The electrical conductivity increases sharply on compression. The existence of phases ranging from fluid to crystalline has been explored by G. S. Emch and others⁸ in terms of algebraic quantum theory; it would be of interest to see applications of this theory to quasi-crystalline phases. Bak⁹ has applied the Landau theory of phase transitions to the icosahedral structures.

One of the characteristics of these icosahedral structures is now recognized to be a conflict, of a kind foreseen by, for example, A. Janner¹⁰ and D. R. Nelson¹¹, between the requirements of local and long-range ordering. (These ideas go back to those of G. Toulouse *et al.* on the topology of glasses.)

Questions of crystallography in two and three-dimensional non-Euclidean manifolds now arise. We have already seen beautiful examples of crystallography in two-dimensional manifolds in the series of papers by S. Andersson and colleagues¹² on periodic minimal surfaces; as there is a third dimension of the world of ordinary experience into which planar patterns may be curved, these provide a way of acquiring experience before trying to make sense of three-dimensional non-euclidean manifolds. But J. F. Sadoc and R. Mosseri¹³ suggest that there is an ideal structure, curved into a fourth dimension which, by disclinations, can be flattened back into ordinary space to give an icosahedral phase. This idea has still to be examined carefully, but we may well be seeing the emergence of 'non-euclidean crystallography'.

Another approach to the interpretation of the experimental results is in terms of projections onto three dimensions of six-dimensional regular crystals. N. G. De Bruijn¹⁴ (for two dimensions) and P. Kramer¹⁵ (for three dimensions) before Shechtman's discovery; and V. Elser¹⁶, and R. K. P. Zia and W. J. Dallas¹⁷ afterwards, have derived the Penrose pattern (a non-periodic tessellation of space by acute and obtuse rhombohedra of two kinds) by projection of a primitive lattice in six dimensions. The resulting pattern was called¹⁸ a 'quasi-lattice' because any vertex can be given integral indices with respect to six axes (three redundant) and corresponds to Frank's consideration of the usual hexagonal lattice (with four axes, one redundant) as the projection of a four-dimensional lattice¹⁹. The pattern is characterized by the algebraic integers which are the golden number, $\frac{1}{2}(1 + \sqrt{5})$, and its reciprocal. As the real space pattern thus involves two irrationally related steps in the same direction, the diffraction pattern resembles the convolution of these two lattices. In the light of Coxeter's discussion of zonohedra as shadows of higher-dimensional polytopes²⁰, are we back with Plato²¹ looking at shadows of reality cast on the wall of our cave?

In the icosahedral structures being

investigated spatial ordering is imperfect, but ordering of the directions of nearest-neighbour bonds persists over long distances. The three-dimensional Penrose pattern can be converted into a nematic texture by leading threads across each tile from face to face. There are 15 directions of these threads, corresponding to the 30 faces of the rhombic dodecahedron which is the basic element of the geometry of the pattern, and which connect pairs of opposite faces. Six layers each containing five sets of threads would be strongly expressed²². The construction resembles the rod packing described by M. O'Keefe and S. Anderson²³ where rods run in the four [111] directions of a cubic structure. In the icosahedral nematic texture, icosahedra connected by edges might represent thread directions²⁴; dislocations would involve line defects²⁵.

So we now have on the cards a number of theories and partial explanations of the icosahedral structures (though D. Levine and P. J. Steinhardt²⁶ suggest that other symmetries may also be possible) of which the following ingredients may have to be dissected and combined:

- Multiple twinning²⁷.
- Hierarchical packing of icosahedra³.
- Bond-orientational order²⁸.
- Three-dimensional packings of the Penrose type, probably with disordering^{18,26}.
- Icosahedral nematic ordering²⁴.
- Hypergrid descriptions and bounded projections of n -dimensional lattices (refs 14–16, 29, 30).
- Incommensurate (modulated) structures¹⁰.
- The flattening into ordinary euclidean space of structures which appear more perfect in a curved metric¹³.

A whole string of questions has arisen and will have to be answered before the observations now to hand are understood. What is the ideal complete ordering of the icosahedral structure, and is it really the three-dimensional analogue of the Penrose pattern? What is the actual quasi-unit-cell edge? What is the nature of the disordering from this ideal structure? And what does it mean physically that a three-dimensional structure should be the (bounded) projection of a structure of higher dimensionality, or even the result of the flattening of a structure curved out of normal space?

Further ahead is another set of questions. What are the requirements leading to the formation of icosahedral phases? Are there others with similar kinds of order? Already there are reports of degrees of order for the Al/Mn material intermediate between the icosahedral and the crystalline. How can the structure best be handled mathematically, in particular for the calculation of structure factors for comparison with experiment after allowance for double-diffraction effects? And what are the physical properties we might

expect from materials such as these?

Let us enjoy a wide-ranging bout of speculation before the definitive answers emerge from crucial observations. For it is too rarely that we see such a stimulating experiment as Shechtman's¹. □

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Animal behaviour

The serpent's seductive scent

from Daniel I. Rubenstein

ANIMALS are not always what they appear to be. Insects often blend into the foliage by looking like twigs or leaves; small hawkmoth caterpillars transform themselves into formidable snake-like creatures by inflating their heads to reveal large eye-like markings; and palatable butterflies, such as the vicory, can so closely resemble noxious species, such as the monarch, that they reduce their risk of being eaten without incurring the cost of acquiring and maintaining toxic substances. Clearly, deceit can help in fooling predators. Recent research, including that described on page 59 of this issue¹, shows that it can be just as useful in duping conspecific competitors.

During the past few years it has become clear that even within populations, marked differences exist in the way individuals compete for reproductive opportunities. Both theoretical^{2,3} and empirical⁴ studies have shown that the reproductive success of males adopting alternative breeding strategies can sometimes equal that of males displaying the typical form of behaviour of a species. For this to be the case, the alternative strategy, which frequently entails forgoing immediate breeding opportunities, must compensate by avoiding some of the costs and risks associated with the typical strategy. Success usually comes by copulating surreptitiously, with perhaps the best form of stealth being to adopt the guise and behaviour of females.

Such female mimicry occurs commonly in bluegill sunfish^{5,6}. In this species the dominant breeding males are large and light in colour — they build nests, drive other light-coloured males away, actively court females and provide parental care.

Other males, however, mate by parasitizing spawning pairs. These individuals are smaller, look like the more darkly coloured and vertically barred females, and simply sidle up to the spawning pair and release sperm as the female deposits her eggs in the nest of the dominant male.

Mason and Crews¹ here show that serpents have added a new seductive twist to female mimicry. Normally, mimicry enables subordinate males to hide from aggressive dominants and thus gain access to receptive females. In the garter snake, however, female-mimicking males are more than just wolves in sheep's clothing.



Male garter snakes, *Thamnophis sirtalis parietalis*, rub their chins along the backs of females during courtship. This behaviour is a specific bioassay for the snake's attractiveness pheromone. Here, three males are courting a female while a fourth has become confused and is courting a 'she-male'. Photo by D. Crews.

Not only do they increase their chances of mating with the receptive female by donning her scent, but they actually seduce, and lure, normal males away from her.

In garter snakes, courtship takes place in writhing mating swarms, or balls; each female is courted by many mates (see figure). Mason and Crews report that many of the mating swarms they studied lacked females; instead, one of the males was being courted by the others. These courted males, or 'she-males' are morphologically indistinguishable from true males, and have higher levels of testosterone and lower levels of oestrogen than true females. Yet these she-males have active female-like pheromones: the pheromone from the skin of she-males is as effective in eliciting courtship from males in experimental arenas as is the pheromone extracted from the skin of females. She-males, however, are not as sensuous as true females. When males are given a choice between a female and a she-male, only about 20 per cent of the males court the she-male. Nevertheless, she-males have a tremendous reproductive advantage. When groups of males and she-males are allowed simultaneously to court an attractive female, approximately 70 per cent of the trials end with matings by she-males. Casual observations of mating balls composed of all three classes of snakes indicated that the she-males actually seduce some males and entice them to court the she-male instead of the normal female.

So far only the seductive effect of she-males on 'he-males' has been examined. Such interactions would have predominated in natural populations when she-males first appeared and were the rarest sexual form. Given the reproductive advantage that she-males show in such interactions, the rapid proliferation of she-males seems inevitable. The effect that she-males have both on other she-males and on true females should be investigated. Do she-males interfere with the reproduction of other she-males to an even greater extent than they do with males? Do females treat she-males differently from he-males? Also, are there ecological factors that limit the viability of she-males? If behaving like a she-male truly entails no costs, however, then an evolutionary problem emerges: why aren't all males she-males? The answers to these questions will be necessary for a complete understanding of the phenomenon Mason and Crews have described. □

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THIS engraving of an American white pelican by John James Audubon is included in the reissue of six engravings of North American birds and in an exhibition at the British Museum of Natural History. Both mark the bicentenary of Audubon, North America's most famous naturalist. His *Birds of America*, consisting of 435 hand-coloured plates, was first published in London between 1828 and 1839. Audubon travelled over the whole United States, painting life-size pictures of native birds, many never described before. The plates were engraved on Double Elephant (991 × 673mm) copper sheets and one edition of 200 copies was printed. Most of the plates were destroyed but the American Museum of Natural History owns 12, six of which have now been restored. The money raised by selling the new issue will finance an Audubon fund at the American Museum of Natural History. The exhibition at the British Museum of Natural History, entitled "Drawn from Life", runs from 3 July to 28 September, 1985.

Stellar evolution

Brown dwarfs and hidden mass

from R. J. Tayler

Is it possible that much of the hidden matter in the solar neighbourhood and elsewhere in the Universe is composed of brown dwarfs, low-mass subluminescent stars that do not become hot enough for nuclear fusion reactions? Until recently no brown dwarfs had been identified. Now infrared observations suggest that the star Van Biesbroeck 8B is such a brown dwarf. On page 42 of this issue L. A. Nelson, S. A. Rappaport and P. C. Joss support this identification by providing detailed calculations of the possible structure and evolution of this star.

It is generally agreed that stars whose masses are less than about a twelfth of a solar mass, the precise limit depending on the chemical composition and on some uncertainties in the physics of stellar interiors, do not attain a high enough central temperature for a useful release of nuclear energy. As a result they have only a short life as stars with significant luminosity, whereas stars whose masses are just above the limit have a very long main-sequence lifetime. The process of star formation is complex and not very well understood; it is not clear whether large numbers of stars will form below the mass limit, although some studies of the fragmentation of gas clouds have suggested that the minimum stellar mass formed might be significantly smaller. If that is true, a substantial amount of matter in the Universe could be in the form of these subluminescent stars, the brown dwarfs.

There appears to be hidden mass throughout the Universe. More than fifty

years ago, Oort pointed out that the strength of the gravitational field perpendicular to the disk of the Galaxy in the solar neighbourhood implied a local mass density greater than that provided by luminous matter. Although much interstellar gas has been discovered since then, there still appears to be hidden mass in the solar neighbourhood. In addition, the variation of rotation velocity with distance from galactic centres implies that many galaxies have higher masses than expected. Finally the dynamics of groups and clusters of galaxies indicates the existence of hidden mass on a much larger scale. It is difficult for all of this matter to be baryonic, if the hot big-bang cosmological theory is valid, but some of it certainly can be, so it is of interest to ask whether brown dwarfs are an important constituent of the Universe.

Brown dwarfs must be difficult to detect unless they are close to the Sun and previous discussions of their probable numbers has been based on estimates of the numbers of low-mass luminous stars. For masses close to and greater than that of the Sun, the number of stars in unit-mass range in a given volume of space increases rapidly with decreasing mass. This mass function must turn over at some low mass so that the total mass in the solar neighbourhood is not impossibly large, but the crucial question is whether this turnover occurs above or below the limit for nuclear reactions. Observers are not agreed on this point. G. Gilmore, N. Reid and P. Hewett (*Mon. Not. R. astr. Soc.* **213**, 257;

1985) are confident that the hidden mass cannot be in the form of dwarfs, whereas J. N. Bahcall, P. Hut and S. D. Tremaine (*Astrophys. J.* **290**, 15; 1985) believe that brown dwarfs are the best candidate.

It seems possible that some of these questions will be answered now that brown dwarfs have been observed. For some time infrared observations have provided candidate objects (see, for example, T. O. Boroson, M. S. Giampapa and J. Liebert, *Astrophys. J.* **283**, 758; 1984) but no definite identifications. Now there seems to have been a definite detection in Van Biesbroeck 8B, the companion to another very low-mass nearby star Van Biesbroeck 8A. The identification has been made using infrared speckle interferometry by D. W. McCarthy Jr, R. G. Probst and F. J. Low (*Astrophys. J.* **290**, L9-13; 1985). Now Nelson and his colleagues describe detailed models of the structure and evolution of very low mass

stars, and show that the observed luminosity and surface temperature of Van Biesbroeck 8B are consistent with its having a mass of about 0.06 solar masses and an age of about 2×10^9 years, comfortably less than the age of the Galaxy. They also suggest that the mass of Van Biesbroeck 8A is very close to the hydrogen-burning limit.

It is obviously of great interest to know that brown dwarfs do exist and to have the opportunity to check the models of low-mass degenerate stars against observations. One or two stars do not, however, account for the hidden mass, so observers will be looking for every opportunity to discover further objects to obtain estimates of their space density. As Nelson *et al.* point out, the Hubble Space Telescope and the Space Infrared Telescope Facility may provide such opportunities. □

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Evolutionary biology

Costs of reproduction

from Linda Partridge and Paul H. Harvey

MOST genetic models for the evolution of life-history patterns assume that an organism's reproduction early in life has a negative effect on its later reproduction. Therefore, reproductive rates at all ages cannot be simultaneously maximized by natural selection^{1,4}. One physiological basis for this effect may be that early reproduction diverts resources away from growth and repair, so that survival rates and fertility are subsequently lowered. Different life-history patterns can evolve as a consequence of reproductive trade-offs. For instance, if growth rates are reduced when an organism's resources are channelled into reproduction, but fertility increases with an individual's size, natural selection may favour delayed maturity and hence higher reproductive success at maturity. Conversely, if larger individuals attract the attention of predators, then early reproduction may be favoured.

These notions of reproductive costs and trade-offs provide an evolutionary explanation for the observed diversity of age-specific reproductive patterns, but until recently there has been little empirical testing of the hypothesis. Attempts to demonstrate and measure the costs of reproduction are producing what at first sight seem to be mixed results.

One major category of study examines correlations between early and late reproduction among different individuals (or clones, populations or species) living in the wild or in captivity. Both negative and positive correlations have been found. For example, the overwinter survival rates of female song sparrows (*Melospiza melodia*) that have reared broods of different sizes are positively correlated with brood size⁵.

Similarly, early and late fertility are positively correlated with survival both in and among clones of a planktonic rotifer (*Platyias patulus*)⁶. In contrast, female red deer (*Cervus elaphus*) calving in one breeding season have a reduced probability of survival to the next breeding season and, if they do survive, of then calving⁷. Only 22 of 33 such studies cited in a recent review⁸ suggest that reproductive costs do occur. But correlation is not causation, and the other studies can be interpreted on the basis of a cost of reproduction if a third variable acts simultaneously on early and late reproductive rates. For instance, individuals may differ in size, nutritional status or their fit to the habitats they occupy, particularly in the laboratory. Under such circumstances, rates of early and late reproduction may be positively correlated, because of their common correlation, with a third variable.

Therefore, when investigating costs of reproduction, it is essential to perform manipulative experiments in which other confounding variables are kept constant⁸⁻¹¹. Laboratory studies with male fruitflies (*Drosophila sp.*) provide an example of manipulations that can demonstrate costs to reproduction when the correlational evidence seems to point in the other direction. The natural correlation between longevity and age-specific reproductive activity in males is positive, because both variables are strongly correlated with body size¹². When reproductive activity is manipulated by allowing males access to an excess of sexually receptive females, rates of survival are reduced¹³. Similarly, female *Drosophila* that lay few eggs because they are kept virgin¹⁴,

or are made sterile by high temperatures¹⁵ or radiation¹⁶ have higher survival rates than females reproducing normally. Similar experiments have manipulated clutch sizes of birds in the field and hence the effort expended on parental care. One study on blue tits (*Parus caeruleus*), outstanding for its careful experimental design and large sample sizes, showed that large clutches result in weight loss and increased mortality in female but not male parents, possibly because although both sexes feed the nestlings, only the female incubates¹⁷.

Genetic manipulations of reproduction have also revealed costs. *Drosophila* females selected for high fertility late in life show increased longevity and lowered early fertility^{18,19}, and females with high fertility early in life have low longevity²⁰.

The general conclusion from properly conducted experiments is that reproduction does have costs; genetic variation has been found in the few cases where it has been looked for, thus providing the raw material for the evolution of reproductive trade-offs. Nevertheless, adequate tests of the importance of reproductive trade-offs in life-history evolution will entail experimental work under field conditions. Laboratory studies probably tend to underestimate reproductive costs, because many important features of field conditions, such as competition for food, predators and competitors are usually absent. However, making the relevant manipulations under field conditions can be extremely difficult. For instance, it is relatively easy to alter clutch and brood size in birds by addition or removal of eggs or nestlings, and hence to measure costs of parental care, but manipulation of the rate of egg laying to assess costs of egg production would be more difficult. Although there are few field studies of reproductive costs, only this type of information will allow adequate tests of some fundamental assumptions of life-history theory. □

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Is the AIDS virus recombinant?

SIR — The retrovirus which is the causative agent of acquired immune deficiency syndrome (AIDS) has recently been shown to be distantly related to the C-type retrovirus^{1,4} and rather share nucleotide sequence homology with the visna virus, a slowly replicating and pathogenic but non-oncogenic retrovirus of sheep that is a member of the Lentivirinae subfamily⁵. The phylogenetic tree inferred from the amino-acid sequences of the DNA endonuclease domain of the retroviral *pol* gene product showed that the AIDS virus is evenly separated from human T-cell lymphotropic virus type-I (HTLV-I) and Rous sarcoma virus (RSV)⁶ (our phylogenetic tree inferred from the same domain differs slightly from that of Wain-Hobson *et al.*⁶; the most distant relative is Moloney murine leukaemia virus (M-MuLV) rather than the AIDS virus). Essentially similar phylogenetic trees were also obtained from the comparisons of the reverse transcriptase domain and highly conserved regions of *gag* gene product, although they differ slightly from each other in tree topology.

In the light of these phylogenies inferred from the *pol* and *gag* sequences, the *env* gene products of the AIDS virus and murine mammary tumour virus (MMTV), a B-type retrovirus, show unexpected homology patterns. The *env* gene product comprises an N-terminal highly divergent region encoding a envelope glycoprotein (gp70 in M-MuLV) and a C-terminal conserved region coding for p15(E), known to be membrane-associated and anchor gp70 at the virion surface^{7,8}. A marked homology is seen in the membrane-associated region between C-type retroviruses (in Fig. 1a, only the homology matrix⁹ of pair M-MuLV/HTLV-I was shown). Although MMTV bears close resemblance in endonuclease sequence with RSV^{6,10}, no obvious homology has been detected in the

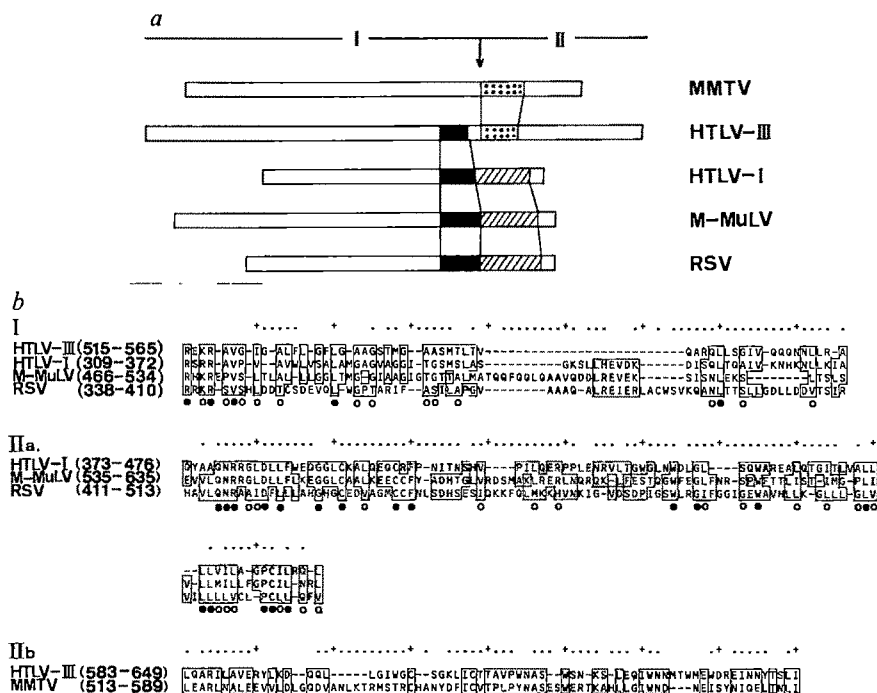


Fig. 2. Alignment of amino-acid sequences of *env* gene products among distantly related retroviruses. *a*, Homology maps along the sequence of *env* gene product. Homologous segments were indicated by vertical lines as well as filled boxes, dotted boxes or hatched boxes. The *env* gene products were tentatively divided into two regions, I and II, as in Fig. 1. *b*, Alignment. For each of the homologous segments shared by different viruses as shown in *a*, their amino-acid sequences were aligned as described previously⁹. Boxed residues indicate identities or favoured amino-acid substitutions among more than half of the viruses. —, Gap; ● and ○, positions that are occupied by identical amino acids and identical or chemically similar amino acids among all the viruses compared, respectively.

env region (Fig. 1b), suggesting that MMTV is a hybrid. Comparisons of the AIDS virus (HTLV-III) with C-type viruses and MMTV revealed unexpected homologies in the membrane-associated region. Evidently homology of the AIDS virus with other viruses drastically changes at a point around amino-acid positions 566–582 (HTLV-III¹ of the *env* gene product). In the regions before this point, the AIDS virus exhibits homology with C-type viruses, but not with MMTV (Fig. 1c). In contrast the AIDS virus shares homology with only MMTV

beyond this point (Fig. 1d). These results are summarized in Fig. 2a and alignments of the homologous regions are shown in Fig. 2b. This mosaic structure of the AIDS virus strongly suggests that genetic recombination occurred between a member of C-type virus and a B-type virus related to MMTV during evolution, generating a novel type of retrovirus, the AIDS virus.

Our proposal that the AIDS virus is a recombinant of B- and C-type retroviruses is consistent with evidence^{2,14} that both the AIDS virus and MMTV use tRNA^{lys} as a primer for (–) strand synthesis instead of tRNA^{pro} used by other mammalian retroviruses, and that they contain very similar polypurine tracts. To confirm further, we searched amino-acid sequence similarities between the AIDS virus and MMTV beyond the membrane-associated region. Somewhat less extensive but significant homology has been detected (positions 712–742 of HTLV-III *env-lor*¹ and 239–269 of MMTV LTR ORF¹³). Their amino acid sequences

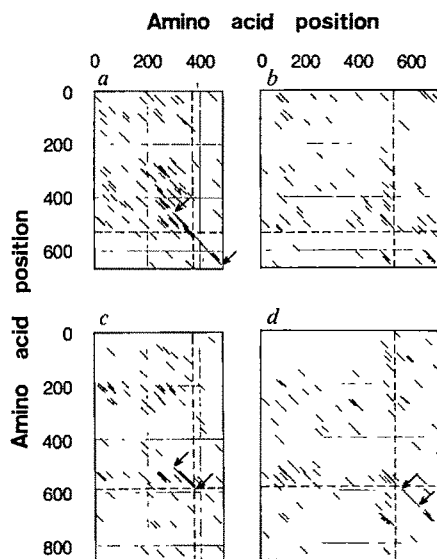
VNRVRQGSPLSFQTHLPPIRGDFRPEGIEE (HTLV-III)

VNGYKVLRYSLPFRERLARARPFWCMSQEE (MMTV)

share 32% homology with the probability of occurrence by chance of less than 2.2×10^{-5} .

It has recently been demonstrated that sequence homology and morphologic similarity exist between the AIDS virus and visna virus⁵. Direct sequence comparison of these viruses together with

Fig. 1 Homology matrix comparisons of amino-acid sequences of *env* gene products of four retroviruses. 1, M-MuLV¹¹ (ordinate) against HTLV-I¹² (abscissa); 2, M-MuLV against MMTV¹³; 3, HTLV-III¹ against HTLV-I; 4, HTLV-III against MMTV. The homology matrices were calculated by the method described previously⁹. Each diagonal line indicates a segment 25 residues long that shows similarity above a threshold level which corresponds to the degree of similarity that would occur by chance with a probability of less than 1.0×10^{-5} . Arrows indicate regions where significant sequence similarities were observed; per cent homology and the probability of occurrence by chance are respectively 40% and $<10^{-9}$ for 1; 37% and 7×10^{-9} for 3; 37% and 4×10^{-7} for 4. No significant similarity was observed for 2. Since HTLV-III exhibits a mosaic pattern of homology with HTLV-I and MMTV, the *env* gene products were tentatively divided into two portions



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MMTV will be of particular interest in relation to our present proposal.

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Prediction of protein structure from sequence

SIR—In a recent News and Views article, Stephen Harrison¹ urges caution “in predicting aspects of protein structure from sequence, what we need to recognize are entire domains or significant sub-domains—not just elements of secondary structure”. He also points out “The poor correlation between local sequence and secondary structure has been aptly demonstrated by Kabasch and Sander², who show that the same pentapeptide in different proteins has fundamentally different backbone configurations in each”.

I wish to point out two items which should caution the reader against accepting such advice without question.

In our original paper on the prediction of protein secondary structure³ (also see ref. 4), it was pointed out with great care that any specific sequence, for example five residues, will assume a conformation dependent upon the neighbouring sequences on both the amino and carboxyl termini until a sequence is reached, of usually four residues, which will either change conformation (β -turn), or will be unable to maintain a secondary structure [$\langle P_{\alpha} \rangle$ or $\langle P_{\beta} \rangle < 1.0$]. Thus it is an expected observation, rather than a novel finding, that a pentapeptide, joined to different sequences may assume dissimilar conformations. Thus the Kabasch and Sander² discovery, used to discredit predictive schemes, reaffirms rather than discredits the predictive algorithm. Harrison's¹ emphasis on domain structure rather than secondary structure to evaluate biological functional units has been expounded with great elegance by Rossman⁵ and is a well accepted theme. However, domains are constructed by in-

teractions of secondary structures; so let us not put the cart before the horse. To understand the formation of domains one must know the secondary structures first. Thus before one has a complete three-dimensional structure of a protein, derived from X-ray crystallographic studies, it can be extremely useful to predict secondary structures and look for homologies with other proteins to find interesting relationships.

I agree with Harrison¹ that the paper of Kabasch and Sander² should be carefully read as it displays how a false conclusion can be drawn from an interesting fact.

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On the possibility of inductive probability

SIR—We here make a second attempt to explain the reason for our hesitation in accepting the Popper-Miller thesis¹. We agree with all of their mathematical relations. Our problem is with the interpretation. We offer again two reasons and frame them here without using the negative signs of our first letter², which appear to have drawn excessive attention to themselves.

First, consider the (correct) result for $p(e) < 1$

$$s(h \leftarrow e, e) \equiv p(h \leftarrow e|e) - p(h \leftarrow e) < 0 \quad (1)$$

Popper and Miller identify the ampliative (or “loosely speaking inductive”) part relative to any evidence, e of any hypothesis h , with $h \leftarrow e$ given e . They infer from (1) that that part of h which goes beyond e is always countersupported by e . (We use their wording in both these sentences.) Our difficulty with this is that $s(k \leftarrow e, e) < 0$ for all values of k and in particular for $k = h$ and $k = \bar{h}$. One might have expected that if the inductive part relative to e of h is countersupported by e , then it would at least be possible for the inductive part relative to e of $\bar{h}$ to be supported by e . But on Popper and Miller's interpretation the inductive part relative to e of both h and $\bar{h}$ is always countersupported by e . This suggests to us that there is something astray with this interpretation, and it led us to observe in ref. 2 that a different definition of terms or some restriction on their interpretation seems to be required. Our interpretation of (1) was explained in our comment on our theorem A, and is rather simple: Since $p(h \leftarrow e)$ still allows $\bar{e}$ to occur, (1) shows that this probability is reduced if $\bar{e}$ is ruled out.

Second, one can understand the import-

ance of discussions of the extent to which 10 or 20 supportive occurrences e support a hypothesis h as a general statement, as is done in statistical theory. If a penny comes up “heads” 10 times ($e_1, e_2, \dots$) is it a penny with two heads (h)? There is a structured relation between e and h . It is, however, difficult to see how statements about induction can go very far if they are based only on probability algebra which leaves h and e as arbitrary propositions. In our letter we noted that the arguments in this correspondence had used only probability algebra, and that they involve the handicap of being independent of any inferential relationship between e and h .

Mathematically speaking, we would say that the support function $s(x, y)$ is indeed useful, as we noted in our comments on our theorem B. It is its division into the suggested “deductive” and “nondeductive” or (loosely speaking) inductive components (and particularly, the interpretation of the letter) that has given rise to our difficulties.

We are grateful to Karl Popper and David Miller for the courtesy of some private exchange of opinions.

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The form of maps in the brain

SIR—Further to Professor Ettlinger's pertinent comments on flying fox somatotomy¹, may I suggest that, if we accept the approach of metric tensor modelling for cortex as well as cerebellum², there would seem to be no requirement for any particular coordinate reference frame, provided that the sensorimotor connections to the central nervous system always preserve the tensorial relationships. Admittedly, my personal interpretation of the metric tensor model leaves me asking why we (and presumably some other vertebrates) need to construct a cartesian four-dimensional space-time at all.

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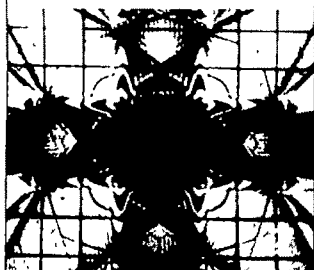
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Selfish DNA and the origin of introns—Correction

IN this piece of Scientific Correspondence (by T. Cavalier-Smith, *Nature* **315**, 283-284; 1985) the word ‘not’ was omitted from line 14 on page 284. The sentence should begin ‘But nuclear tRNA does not depend on specific intron sequences ...’

David B. Williams

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in Materials Science**



David B. Williams

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Approaching the past

Colin Renfrew

Archeological Explanation: The Scientific Method in Archeology.

By Patty Jo Watson, Steven A. LeBlanc and Charles L. Redman.

Columbia University Press: 1984. Pp. 309. \$26.50 (United States), \$34.50 (elsewhere).

Anthropological Archaeology.

By Guy Gibbon.

Columbia University Press: 1984. Pp. 455. \$29.50 (United States), \$38.50 (elsewhere).

ONE of the principal objectives of the "New Archaeology" of the late 1960s was to be "scientific". This meant less the use of various technical resources drawn from the natural sciences, such as radiocarbon dating or trace element analysis, than the attempt to place the reasoning behind the subject on a systematic basis.

The most persuasive of the New Archaeologists was Lewis R. Binford. Together with the first generation of his students, he sought to enlarge the scope of archaeology by asking new questions of the archaeological record, by developing techniques (such as efficient sampling strategies) which could answer specific questions once these had been clearly formulated, and by attempting to find more satisfying explanations for past events. These explanations were framed in terms of culture process: that is to say, in terms of general perceptions of the way various aspects of human societies have tended to operate. Binford and his colleagues were critical of many of the more traditional historical explanations, which were seen as frequently particularistic, tending merely to illuminate the case in question with a wealth of circumstantial detail, and often explaining the major events of the past in terms simply of the intentions of the principal actors, the Great Men of history.

The first generation of New Archaeologists emphasized the need to make explicit the bases of archaeological inference, to avoid judgements based on purely subjective assessment, and to find ways of testing hypotheses about the past, wherever possible by the use of fresh data. All of this added up to what the late David Clarke rightly termed the "loss of innocence", implying the development of a critical self-awareness of the nature of the discipline. The subject has continued to develop steadily since those early days, and has indeed met some of the hopes of those advocating an "explicitly scientific approach".

This phrase, *An Explicitly Scientific Approach*, formed the subtitle of the fore-runner of the first of the books under review, which was originally entitled *Explanation in Archeology* and published in 1971. It was an important book, seeking in a systematic way to apply the insights offered by the philosophy of science to aid archaeologists in making their subject

more scientific. This should, one might think, have been a royal road to success, but it proved instead something of a detour over philosophically shaky terrain towards rather sterile ground. The authors were open to criticism (as some reviewers indicated at the time) for following rather too readily the view propounded by the philosopher of science Carl Hempel. Hempel argued that nearly all explanation, whether in history or in the sciences, rests ultimately on the formulation of universal laws. His Deductive-Nomological (D-N) model of explanation offered an ideal to which all sciences (even historical ones) were thought to aspire.

Rather than follow this portentous mentor, the authors would have done better to ask what, in various good explanations known to them in the field of archaeology, is in fact the underlying structure of the explanation, rather than what it *ought* to be. What was clear to several archaeologists in 1971—that Hempel was an unsatisfactory guide—is clear to many philosophers of science today. Within that discipline what was once a widely accepted commonplace, the D-N model of explanation, is now seen as naive and problematic, and of much narrower application.

The present work, published 13 years later, has certainly learnt something from its initial *mésalliance* with a dogmatic philosophy of science. Different forms of Cover Law model are discussed in the first chapter, entitled "The Logic of Science, the Nature of Explanation and Archeology as Science". And happily the book moves on from these matters to talk about various aspects of archaeology in an informed and stimulating manner. Systems theory and ecology are discussed in succeeding chapters, and then the archaeological record and the design of archaeological research, and archaeological analysis, with a final chapter entitled "Archeology and Society".

Unfortunately, however, the authors have not been able to escape from the influence of Hempelian thought, and the tone of the book is firmly established in the first chapter. I find their view of science, as presented there, insensitive to the problems associated with explaining the behaviour of highly complex systems, as most living systems are. The D-N framework and statistical laws may work

well for physics, or for fields such as thermodynamics or psephology where very high numbers of unitary individuals make a probabilistic approach appropriate. But I doubt whether a molecular biologist or a meteorologist would recognize his or her science in these formulations. In fact the formal analysis of very complex systems has developed considerably in recent years, and we can begin to see that, even in the hard sciences, the trajectory of the system (that is to say its past history) as well as its present state may be relevant to its future behaviour. Fields such as catastrophe theory and the study of non-equilibrium systems are influencing archaeology as well as other sciences. The criticism to be made is therefore not necessarily the familiar one that human affairs cannot be treated scientifically, but rather that the vision of science offered here is not well developed for the explanation of the behaviour of highly complex systems.

In general, I think that the philosophy of science has served archaeology very badly over the past 10 to 15 years. The aim of being scientific, of setting explanations within an explicit framework of coherent theory, of developing a valid epistemology, is incontestably worthy. But so far most of the contributions from philosophers have been of a Procrustean inflexibility, seeking to impose on the subject under study, in this case archaeology, a series of preformulated precepts which have later themselves been shown to be of limited value. It now seems to me that to be a good philosopher of archaeology, and to produce insights into the nature of explanation in archaeology, it may pay to be a good archaeologist first.

This book, alas, continues to suffer from some of the defects of its predecessor: it tells us first how archaeology would look if it were scientific, and then tries to make the image fit. This is not only poor archaeology: it is poor philosophy of science. Perhaps it is even poor philosophy. I find it disconcerting that the first chapter, which attempts to define the nature of explanation in archaeology, and does so in terms of universal laws, offers very few examples of this kind of explanation in archaeology, and none at all which relates to human behaviour.

The book goes on to discuss several aspects of archaeology in an interesting way. It is invariably well-informed, and certainly worth reading. I share its general objectives: archaeology did need to be set on a better basis, and has benefited from the serious discussions about its nature over the past 15 years. But I am doubtful of the general validity of the view of science which is offered here.

Anthropological Archaeology is a new work which, likewise, sets out to tell us what science is, and what the philosophy of science is, before telling us very much about archaeology. It is serious, and

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rather long. But although richer in examples (and illustrations) than *Archeological Explanation*, to my mind the author does not always understand his own examples very well, giving abbreviated summaries of important work which do not always catch the essence of the original. For instance I am not satisfied that the outline of the "wave of advance" model for the spread of agriculture (pp.262-265) shows how a delightfully simple dynamic formulation is derived from a very plausible set of explicit assumptions and initial conditions.

There are also some confusions, which are reflected in the glossary. "Diachronic analysis" — which implies analysis involving the passage of time and contrasts with synchronic analysis — is defined as "in the systems approach, analysis that is concerned with the mechanisms rather than the structural end products of changing subsystem relationships". This seems needlessly complicated. Survivorship curve is defined as "estimates of the percentage of individuals in subgroups of a theoretical population of 100 people that will still be alive at the end of a five year period". (Query: why specify five years?) "Tool kits" are said to be "spatial associations of archaeological materials that are assumed as a working hypothesis, to be meaningfully related to a specific task". That lacks precision, as well as clarity, and could apply as well to Stonehenge or the Pyramids of Egypt as to the subject intended.

Anthropological Archaeology does cover a number of issues seriously and thoughtfully. Its intentions, like those of *Archeological Explanation*, are thoroughly honourable. Many processual archaeologists, myself included, would share the author's aim of treating the subject in a thorough and systematic way. But this book does not convey the clarity of thought which anthropological archaeology should employ.

These two works reflect the real concern of the contemporary archaeologist to come to terms with the epistemological basis of the subject, and to develop coherent theoretical approaches. For this they will command respect. But neither they, nor the philosophers of science on whom they rely, adequately convey the lesson that different kinds of systems behave in different ways, and that complex systems can only rarely be described, and their behaviour explained, in terms of universal laws which go much beyond the obvious, the banal or the very general. The moral seems to be that the philosophy of archaeology, or of any specific science, is too important a subject to be left to the dictates of the generalist philosopher of science. □

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The shape of things to come

John D. Barrow

Mathematics and Optimal Form. By Stefan Hildebrandt and Anthony Tromba. W.H. Freeman: 1985. Pp.215. £20.95. To be available in the United States outside the Scientific American Library later this year.

WHAT do dirty washing and the design of the Great Pyramid have in common? The sort of tenuous "connection" that might generate an entire BBC television series perhaps? But there really is a connection between soap-suds and skeletons, honeycombs and Hero, the Queen of Carthage and the Olympic stadium in Munich. All are linked by the intriguing subject of optimal mathematical form, which was first discussed as a controlling factor in the natural world by D'Arcy Thompson. Here, two mathematicians who have made important contributions to the calculus of variations and minimal surfaces have produced a well-written and profusely illustrated study of the influence of extremum principles in nature.

This subject has a curious pedigree. The first modern variational principle, the so-called "Least Action Principle", was framed by Moreau de Maupertuis in 1744, although "minimum" principles had been employed by Hero to demonstrate the law of optical reflection nearly 1,700 years earlier. Maupertuis was influenced by Leibniz's doctrine of the "best of all possible worlds" and sought to overcome the defect that it appeared to be a statement of comparative reference about a unique entity. How can you say the observed laws of motion are optimal when you know of no others? Maupertuis pointed out that a certain combination of mass, velocity and the distance traversed was always minimized by the actual behaviour of a dynamical system, a requirement equivalent to imposing Newton's laws of motion. Maupertuis claimed that this displayed precisely in what sense the laws governing our world were optimal, and the inferior alternative worlds were those whose laws of motion gave paths of non-minimal action. This provided the basis for his teleological interpretation of nature. He even regarded the Least Action Principle as the most powerful proof for the existence of God. (A hundred years later a few theologians tried to maintain the doctrine of special creation in the face of fossil finds by claiming that the "failed" species revealed there were the products of the paths of non-minimal action!)

Hildebrandt and Tromba include much of the history of the calculus of variations and the appearance of optimization problems in the ancient world, for example the challenge to Dido of Carthage that she

could colonize only the area of land enclosed by the skin of an ox. Having split the skin into the thinnest strips possible, what is the optimal shape to enclose? The core of the book explains aspects of mathematical research into minimal surfaces and a variety of optimization problems involving processes occurring on unusual geometries and topologies. The results are illustrated by examples from biology, architecture, foams and bubbles, fluid flow and crystallography. Even professional mathematicians will find much that is new to them in these chapters — besides providing grist for popular lectures, they might provoke inclusion of options on the rigorous theory that underlies the subject in undergraduate mathematics courses.

Physicists will detect several absent topics that deserved inclusion in a book of this type. For example the discussion of geodesics in general relativity is too brief to be useful and misses the key contrast between Euclidean space and Minkowski's space-time: in Minkowski space-time there exists a longest world-line joining any two points, but no shortest one. The opposite is true in Euclidean space.

Altogether this is a most enjoyable book — a beautiful illustration of the interplay between mathematics and the natural world, interestingly and carefully written. □

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Spirits of Victorian England

Ivor Grattan-Guinness

The Other World: Spiritualism and Psychical Research in England, 1850–1914. By Janet Oppenheim. Cambridge University Press: 1985. Pp.503. £25, \$44.50.

ENGLISH Victorian thought continues to fascinate historians, not only for the quantity and quality of the work achieved but also for the range and complexity of philosophical positions attendant upon it. Not for them the quick lazy positivism of our modern science: this was natural philosophy, the study of nature in all her physical manifestations, and with the ontological and religious aspects set in place. One feature of these endeavours was the prominence accorded to study of the mind

and its means of communication, both in orthodox contexts and in the realms of "spiritual" activity. "The word *spiritual* is one of the most difficult and important terms in nineteenth-century thought", wrote F.M. Turner in his *Between Science and Religion: The Reaction to Scientific Naturalism in late Victorian England* (1974), and various historians have recently developed this theme in the context of psychical research, especially B. Inglis in *Natural and Supernatural* (1977). Now we have the best study to date.

Janet Oppenheim, a young American scholar about whom the publisher tells us nothing, has ransacked the publications of the period, a wide range of manuscript collections and the relevant secondary literature to produce not only another account of the rise of psychical research in the late nineteenth century but also a detailed statement on the varieties and place of spiritualism during that period. Her book falls into three parts. "The Setting" describes in two short chapters the activi-

ties of various mediums and the wide spread of spiritualist publications and societies in England at that time. "A Surrogate Faith" explains with great lucidity the plurality of positions embraced by spiritualism — the survival of spirit after death was the common assumption, but a wide range of views was adopted by different groups — and then the complicated relations between spiritualists, the principal workers within the Society for Psychical Research (SPR), which was founded in 1882, and theosophists. After a welcome suite of portraits and illustrations, there then comes the excellent third part, unhappily entitled "A Pseudoscience", in which the places of psychical research and spiritualism are described for three areas of science: in turn, psychology and phrenology (the latter a special *Scottish* interest, as the author might have pondered); evolution and medicine; and physics.

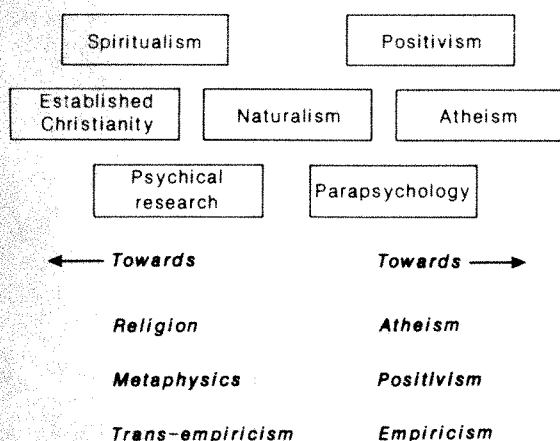
Four hundred pages of beautifully composed text are followed by 90 pages of end-notes, full of interesting information and references; however, they lack proper cross-references, so that it can be hard to find the full details of an item.

The richness and complications of Victorian thought cannot be fully conveyed in a review, although the figure (see left) and its attendant commentary include some further explanation. I focus, therefore, on the last part of the book, which is of most interest to scientists today. Sensibly, Oppenheim chooses to present the variety of positions by describing *seriatim* the views of major figures concerning spiritualism, religion, psychical research and their specialist areas of knowledge: wholehearted spiritualists such as Alfred Russel Wallace and William Crookes; men of a more cautious or vacillatory stance, such as G. J. Romanes, J. J. Thomson and Oliver Lodge; and those typified by Henry Sidgwick and his wife Eleanor, who could not subscribe to any established religious position (and in their cases also became opposed to spiritualism). The only group omitted from the survey are logicians, which is a pity, since at that time logic and psychology were closely linked. Augustus de Morgan and his wife are mentioned, but they lack the company of Mrs Boole, propounder of her late husband's algebra and also founder council member of the SPR; Lewis Carroll and John Venn; and W. E. Johnson, brother and mathematical assistant to the first research officer of the SPR, Alice Johnson.

The intertwining of the study of nature, the belief in survival and the pursuit of the paranormal that characterized a large part of Victorian thought is re-created in this book to an extent not previously achieved by an historian. Oppenheim has produced an altogether superior study. □

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REPRESENTATION of the spectrum of philosophical positions adopted by Victorians on religious and scientific questions. The boxes are placed on separate lines merely for convenience: the linear form adopted is a considerable over-simplification (so that, for



example, theosophy cannot fairly be indicated on it), but it conveys some impression of the distinctions and overlaps. "Naturalism" was a term used at the time to describe views which usually preferred Nature to God, facts to belief, reason to faith, and

matter to spirit. To one side lies "psychical research", referring to views normally held by leaders of the SPR (which, however, held no *corporate* opinion); to the other is "parapsychology", used in the narrow sense to refer to the experimental, laboratory-orientated studies associated from the 1930s on with J. B. Rhine and his followers. The linear diagram, then, also has a chronological overtone, from left to right. For further details see *Research in Parapsychology* 1982 pp. 283–304 (Scarecrow Press, Metuchen, NJ, 1983).

Elements of disaster

John B. Whittow

Volcanic Hazards: A Sourcebook on the Effects of Eruptions. By R.J. Blong. Academic: 1984. Pp.424. \$66, £49.50.

IN A period when such environmental disasters as drought in Ethiopia and flood in Bangladesh make headline news, it has now been generally acknowledged that many Third World communities have become so "marginalized" that the slightest aberration in their local atmospheric or geophysical systems may trigger a disaster. Records suggest that there has been no increase in either the magnitude or the periodicity of hazardous events, but death tolls continue to rise.

Bare statistics disguise a significant trend, however, for while the loss of life from natural disasters has increased in the developing world, deaths in the developed world have fallen. Most observers claim that the dichotomy results from the "technological cushion" enjoyed by developed nations, where scientific knowledge, organized planning and advanced warning systems have combined to alleviate the threat and ease the impact of natural hazards. Critics highlight the distinction between those societies which can choose from a long list of structural and non-structural adjustments and those which have no choice. The latter have limited expertise and local infrastructure, little or no opportunity to insure against disaster, and even if warnings could be disseminated the lack of mobility inhibits evacuation: they must bear the loss of life and property.

Any new publication dealing with natural hazards must be viewed in this light. Increasingly, the value of such books is judged by the degree to which they offer to help reduce the vulnerability of threatened populations, whatever their economic or social status; it is no longer enough simply to catalogue and explain the physical phenomena of natural hazards. One is encouraged, therefore, to discover that *Volcanic Hazards* has this humanitarian concern as one of its three aims. Its other objectives, of chronicling and analysing the effects of the hazard, follow the traditional approaches adopted by the plethora of books on the subject which have appeared over the past decade.

Volcanic hazard research has progressed far beyond such pioneering works as Symons and Lacroix who, respectively, described the famous eruptions of Krakatau and Mont Pelée almost a century ago. Even the modern global syntheses of Bullard¹ and Francis² have rapidly

been overtaken as earth scientists have become more concerned with effects than with causes. Following the valuable work of Walker³ on volcanic prediction, and of Sheets and Grayson⁴, who examined the relationship between volcanoes and human ecology, there has been an urgent need for a single volume that would bring together the mass of literature on volcanic eruptions in a clear and concise form. R.J. Blong has provided such a volume. Here is a well-written "sourcebook" (the author's own subtitle) which not only examines all of the well-chronicled volcanic events from Santorini (1500 BC) to Mount St Helens (AD 1980), but also provides, at the end of each chapter, an appraisal of the impact of volcanic activity on various aspects of the environment.

In Blong's assessment of deaths and injuries caused by volcanic eruptions, several points emerge. For instance,

activity have resulted from starvation and disease. It is a pity, therefore, that while the devastating effects of tephra falls, pyroclastic flows etc. on crops and livestock are critically discussed at local scales, Blong says virtually nothing about the influence of volcanic dust veils on world climates and thus on harvests. Unfortunately, he believes the topic to be beyond the scope of his book.

There are no such omissions in the sections dealing with the impact of volcanic eruptions on buildings, public utilities, communications and economic activity. When it comes to property losses we discover, not surprisingly, that it is the developed world that has borne the brunt. Almost half of the book is devoted to an analysis of the effects on the built environment, and although worldwide case studies are included Blong relies heavily on the Mount St Helens disaster.



Destruction by volcano — *The Last Days of Pompeii*, by Brüllov.

deaths due to volcanic activity are contrasted with those resulting from other natural hazards and it is shown, for example, that the victims of earthquakes outnumber volcanic fatalities by a ratio of 12:1. Of even greater note are the figures indicating that Indonesia suffers no less than 67.3 per cent of the world's volcanic casualties, compared to a combined total for New Zealand and the Mediterranean countries of 2 per cent, despite the fact that all three regions are together regarded as the most volcanically active in the world. The statistics can, of course, be explained in part by the respective population densities of the three areas. But when a developed country such as Japan (just as over-crowded as Indonesia) is taken into account, one has to conclude that Japan's 8 per cent of the total casualties must be explained by its "technological cushion" and its reduced vulnerability to hazard-induced crop losses and subsequent famine. Indeed, in the context of food shortages in the Third World, the chapter dealing with volcanic effects on agriculture is of great importance, in particular because, as Blong shows, since 1600 some 40 per cent of deaths from volcanic

This is a welcome synthesis of information on the 1980 eruption, and one which puts the economic effects in their true perspective. We learn, for example, that local employment actually increased and that the economic impact was less than that of the Boeing recession. One suspects that were it not for the mass of volcanic perception studies generated by Mount St Helens, the valuable chapter on social aspects of eruptions would never have appeared. Like other hazard perception studies, it illustrates the impossibility of producing a regional disaster plan without knowing something of the likely behavioural responses of decision makers as well as of those at risk. Without such knowledge disaster relief funding, evacuation plans and survival rates are difficult to assess.

Because it offers broad outlines for risk assessment, *Volcanic Hazards* will be invaluable to administrators, engineers and planners in developing and developed societies, as well as appealing to all earth scientists concerned with volcano monitoring and hazard delineation. A careful balance has been maintained in the treatment of man-environment relationships, the examples are well-researched, clearly explained and superbly referenced, and the whole is supported by many high-quality photographs, maps, diagrams and tables. This is an elegant volume which satisfies a longstanding need in the literature of natural hazards.

1. Bullard, F. M. *Volcanoes of the Earth* (University of Texas Press, Austin, 1976).

2. Francis, P. *Volcanoes* (Penguin, Harmondsworth, 1976).

3. Walker, G. P. L. *Geol. Soc. Lond. Misc. Publ.* 3, 23-41 (1974).

4. Sheets, P. D. & Grayson, D. K. (eds) *Volcanic Activity and Human Ecology* (Academic, New York, 1979).

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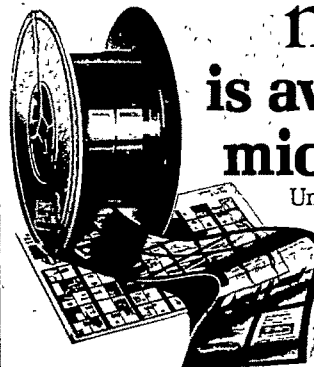
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Superfluidity in neutron stars

David Pines & M. Ali Alpar*

Department of Physics, University of Illinois at Urbana-Champaign, 1110 W Green, Urbana, Illinois 61801, USA

Recent developments have radically changed our understanding of the dynamics of neutron-star superfluids. We review astrophysical evidence for the existence of at least three distinct superfluids inside neutron stars from the observed relaxation of the rotation and spin-down rates of the pulsars Vela, Crab and PSR0525+21, following sudden jumps (glitches) in these quantities. We also discuss the effects of the superfluid interior in the accreting neutron-star X-ray sources.

SUPERFLUIDITY in neutron stars was first suggested^{1,2} before the observation of such stars; the suggestions were as remarkably prescient as the first theoretical proposals of the existence of neutron stars and pulsars³⁻⁵. The discovery of radio pulsars and accreting X-ray sources placed neutron stars firmly in the realm of observational astrophysics. Subsequent accumulation of timing data revealed that radio pulsars experience sudden period changes ('glitches'), and their relaxation following such a glitch is too slow to be explained in terms of the viscous processes of normal matter. These timing observations provided the evidence for the presence of cosmic superfluidity and motivated the theoretical work on the rotational properties of this superfluid⁶. As is well known from laboratory experiments, the rotational state of a superfluid is determined by a distribution of quantized vortex lines, just as in a hard superconductor the magnetic field configuration is determined by a distribution of quantized flux lines. The motion of these vortex (or flux) lines is governed by their interactions with normal matter, including the possible pinning of the vortex lines to preferred sites in an inhomogeneous medium, a phenomenon well known in studies of hard superconductivity. The subject of this review is the fascinating extension of these basic concepts of superfluidity to the largest superfluid systems encountered in nature, neutron stars. Several physical effects peculiar to the dense state of matter in neutron stars play a key role in this extension.

The salient features of the current picture are that: (1) the core superfluid, which comprises the bulk of the moment of inertia, rotates rigidly with the crust of the star despite being in the superfluid state; (2) because of vortex pinning the crust superfluid, which contributes $\sim 10^{-2}$ of the star's total moment of inertia, can be out of equilibrium with the observed crust for timescales of weeks, months and years, and is responsible for a variety of interesting timing behaviour, primarily pulsar glitches and post-glitch relaxation; and (3) the observed post-glitch relaxation times provide a means of estimating the interior temperature of pulsars.

Structure of neutron stars

The details of neutron-star structure are quite sensitive to the still imperfectly understood neutron matter equation of state at densities greater than $\rho_0 \approx 2.8 \times 10^{14} \text{ g cm}^{-3}$, the density of nuclear matter⁷. If the basic hadron equation of state is comparatively stiff (see ref. 8 for a recent review), the cross-section of such a star would be that shown in Fig. 1. Beneath an atmosphere only a few metres thick one finds an outer crust, 0.5-km thick, of increasing density ($7 \times 10^6 \text{ g cm}^{-3} \leq \rho \leq 4 \times 10^{11} \text{ g cm}^{-3}$), containing a solid array of nuclei and a highly degenerate relativistic electron plasma⁹. The inner crust begins at $\rho \sim 4.3 \times 10^{11} \text{ g cm}^{-3}$ and extends a few km until a mass density $\rho \sim 2.4 \times 10^{14} \text{ g cm}^{-3}$

is reached. It contains a highly degenerate superfluid neutron liquid as well as the lattice of increasingly neutron-rich nuclei and relativistic electrons. The quantum liquid interior of the star is expected to consist mainly of superfluid neutrons, with an admixture of a few per cent of superconducting protons and normal electrons. The constituents of neutron stars at densities well in excess of ρ_0 are uncertain (see ref. 10 for a review of some of the possibilities). It is possible that at densities a few times ρ_0 one may find an alternating layer structure involving a pion condensate^{11,12}. Quark matter is not expected¹⁰ because the maximum central densities of neutron stars ($\leq 5\rho_0$) are rather less than those at which such a state would be favoured. Because of the very high thermal conductivity of degenerate matter, the temperature, T , of the star is quite uniform, and is typically $\sim 5 \times 10^8 \text{ K}$ for a young neutron star such as the Crab pulsar. This would seem at first sight to be high, until one realizes that, because of the extraordinarily high densities involved, typical crustal melting temperatures are $\sim 10^{10} \text{ K}$ (1 MeV), whereas degeneracy temperatures for the quantum liquid constituents are still higher (5–50 MeV) and energy gaps for the neutron and proton superfluids may represent an appreciable fraction of their degeneracy temperatures. The crustal material thus resembles terrestrial solids in the liquid helium range, whereas the quantum liquids resemble liquid He in the millidegree range.

Neutron superfluids

The effective interaction between two neutrons is a combination of a strong short-range repulsion and a long-range attraction. At least two distinct pairing states of neutron superfluids are expected: (1) in the inner part of the crust, where the interparticle spacing is greater than the range of the repulsive forces ($\sim 10^{-13} \text{ cm}$), $^1\text{S}_0$ pairing dominates¹³⁻¹⁵. (2) at the higher densities ($\geq \rho_0$) characteristic of the quantum liquid interior, the combination of short-range repulsion and an attractive tensor force leads to a $^3\text{P}_2$ paired superfluid^{13,16}. The transition temperatures are shown in Fig. 2 as a function of density in the star.

Because neutron stars rotate, neither neutron superfluid will be spatially uniform; both contain an array of vortex lines parallel to the rotation axis, of sufficient number that on a macroscopic scale the neutrons will seem to be rotating as a rigid body. As the circulation per vortex is $\kappa = h/2m_n$, the density of vortices per unit area is $2m_n\Omega/\pi\hbar$ to mimic rigid-body rotation (where $\hbar$ is Planck's constant, m_n is the neutron mass, and Ω is the rotation frequency). This vortex density is $\sim 2 \times 10^5 \text{ cm}^{-2}$ for the Crab pulsar ($\Omega \approx 191 \text{ s}^{-1}$).

In the stellar crust, the normal cores of the vortices where the condensate wavefunction decreases to zero either may be pinned to the lattice of nuclei present there or may thread the spaces between them. Because the nuclei also contain superfluid neutrons, whether or not pinning occurs is a matter of comparative superfluid energetics. It is favourable for a vortex line to

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pin to a nucleus if the energy cost per particle to create its normal core, $\sim \Delta^2/E_F$, where Δ is the energy gap for superfluid neutrons and E_F their Fermi energy, is thereby reduced⁶. More precisely, the pinning energy per nucleus is^{17,18}

$$E_p = \frac{3}{8} \left[\frac{\rho_{\text{out}} \Delta_{\text{out}}^2}{(E_F)_{\text{out}}} - \frac{\rho_{\text{in}} \Delta_{\text{in}}^2}{(E_F)_{\text{in}}} \right] V \quad (1)$$

Here 'out' and 'in' denote local values of Δ and E_F for superfluid neutrons outside and inside the nuclei, V is the overlap volume of the vortex core and the nucleus of radius R_N ; the core radius is the coherence length of the outside neutron superfluid,

$$\xi = 2E_F / \pi k_F \Delta \quad (2)$$

Calculations^{15,19} suggest that the pinning layers, in which it is energetically favourable for vortex lines to go through the nuclei, extend from densities $\sim 10^{13}$ to $\sim 2 \times 10^{14} \text{ g cm}^{-3}$. With a pinning energy E_p , the maximum available pinning force per unit length of vortex line is

$$f_p = \frac{E_p(\rho)}{\xi(\rho)b(\rho)} \quad (3)$$

where ρ is the average density characterizing the pinning layer and b is the spacing between successive pinning centres along the vortex line.

The pinning energy and coherence length are determined by the equilibrium nuclear configuration²⁰ and the superfluid energy gap, while the distance between pinning centres depends on the physical circumstances under which pinning takes place. Pinning will be maximal in the strong pinning layers, where E_p is greater than a typical lattice energy, $E_L \sim Z^2 e^2 R_N / b_z$ (b_z is the lattice spacing), so that the pinning force is strong enough to dislodge nuclei from their equilibrium sites in the lattice, and b is comparable with b_z . The transition to weak pinning layers occurs when $E_p \leq E_L$. In the weak pinning layers, the vortex core pins only to those sites through which it passes; the spacing between pinning sites, b , is $\sim (E_L/E_p)b_z$, and the effective pinning energy is reduced to $\sim E_p(E_p/E_L)$ (ref. 18). Finally, where the coherence length, $\xi \geq b_z$, a vortex core can 'pin' to more than one nucleus at a time. Under these circumstances, which are expected in the higher-density regions of the pinning layers, moving a vortex line requires still less change in the pinning energy; one is in a region of superweak pinning¹⁹, in which the effective spacing, b , between multiple pinning sites is far larger than $(E_L/E_p)b_z$.

The proton superfluid

The basic interaction between isolated protons resembles that for neutrons, whereas the density of the proton liquid in the stellar core is some two orders of magnitude smaller than that of the neutron liquid; hence the interior proton liquid will superconduct in a $^1S_0$ pairing state²¹.

Because the critical field, $H_{ci} \geq 10^{15} \text{ G}$, is enormous compared with the field strengths $\sim 10^{12} \text{ G}$ that are found in pulsars, one might expect that the proton superconductivity would have a dramatic effect on the magnetic properties of the neutron star, through expulsion of the flux from the superconducting regions. It does not, because the electrical conductivity, σ , of the normal state is so large that the characteristic time for flux expulsion from macroscopic regions is typically $\gg 10^8 \text{ yr}$ as Baym, Pethick and Pines²² have shown. As the star cools, superconductivity nucleates with the magnetic field present. The London penetration depth is greater than the proton coherence length, so that the flux penetrates the superfluid region in a vortex state similar to that found in laboratory type II superconductors for $B > H_{ci}$.

Core neutron communication

The principal energy source of a pulsar is the rotational energy of the neutrons. How do the core neutrons, which make up the bulk of the neutron star, follow changes in the rotational state of the crust? Consider first the charged particles. The electrons and protons in the interior must co-rotate by electromagnetic

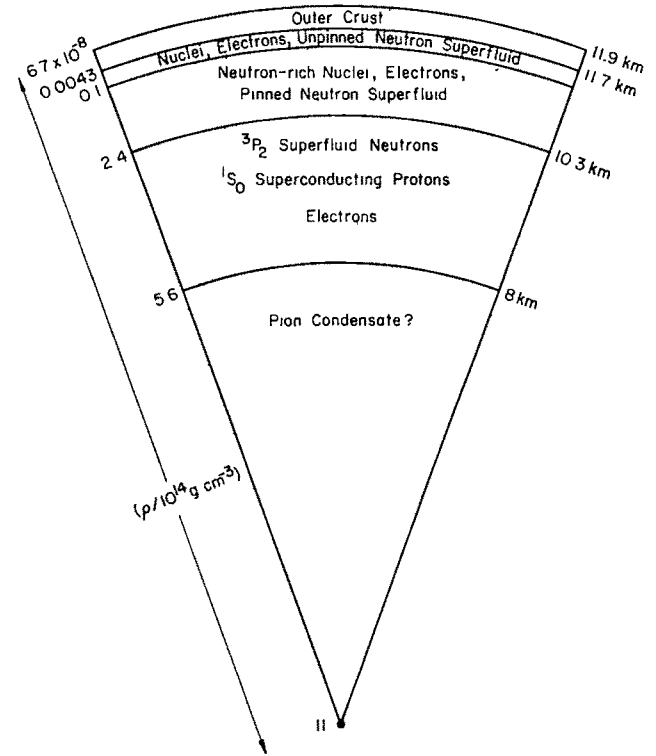


Fig. 1 Cross-section of a $1.4 M_{\odot}$ neutron star, calculated using the Bethe-Johnson equation of state.

coupling, irrespective of whether or not the protons are superfluid (any appreciable differential rotation would give rise to inordinately large magnetic fields). The charged particles in the crust and those in the interior communicate readily through the viscosity of the electrons and/or the magnetic field in the interior. Easson²³ finds that the core plasma responds to sudden changes in the crustal angular velocity with a magnetic spin-up time $\sim 10 \text{ s}$. The Ekman time for electrons is of the same order^{23,24}.

The rotational state of the neutron superfluid is determined by the distribution of its quantized vortices. Any change in the rotation rate of the crust is communicated to neutron vortex cores via electrons and protons. Thus, the dynamical coupling time of the neutron superfluid is determined by the timescale for the scattering of electrons against the normal cores of superfluid neutron vortices. For electron-neutron magnetic moment scattering, the charged particle-neutron coupling time is found to be $\sim 10^7 \text{ s}$. These considerations led to the conclusion that if both the neutrons and the protons in the stellar interior are superfluid, the relaxation time which characterizes the coupling between the crust and the interior superfluid neutrons is a macroscopic time, of the order of years²². This formed the basis for the proposal of the two-component model of crust-core coupling in neutron stars²⁵. More recent work^{26,27} has shown that there are mechanisms of intrinsic magnetization of the neutron vortices that result in a much stronger coupling of the neutron superfluid to the charged particles and to the crust.

Glitches and post-glitch relaxation

The inner part of the pulsar magnetosphere adjacent to the neutron star crust rotates with it. Thus, the observed pulse timing reflects the rotational behaviour of the crust. Sudden increases in rotation rate, termed glitches, have been observed from many pulsars. The Crab pulsar has had two prominent glitches²⁸ with $\Delta\Omega/\Omega \sim 10^{-8}$ – 10^{-9} . The old pulsar 0525+21 has exhibited a glitch of similar magnitude²⁹. The Vela pulsar has had six glitches^{30–32} with $\Delta\Omega/\Omega \sim 10^{-6}$ since 1969, and each of the pulsars 1641–45, 1325–43 and 2224+65 have shown one such giant glitch^{33–35}. The statistics of the Vela-type giant glitches is consistent with the hypothesis that all pulsars have them, although glitches become less frequent as pulsars evolve³⁶.

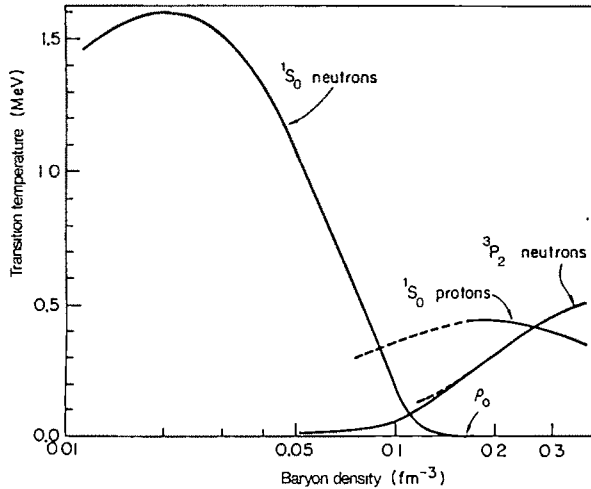


Fig. 2 Calculated superfluid transition temperature as a function of baryon density for neutrons and protons¹⁰. Dashed curves indicate extrapolations to lower densities

Glitches are sudden in the sense that observationally their rise time seems to be less than a day. By contrast, the post-glitch relaxation in the timing behaviour of a pulsar, which has been observed in the Crab, Vela and PSR0525+21, has timescales from a few days to years.

Post-glitch relaxation is determined by the coupling of the crust to the superfluid neutrons. Let us write the torque acting on the crust of a neutron star rotating at angular velocity, Ω_c , as

$$I_c \dot{\Omega}_c = N_{\text{ext}} - N_{\text{int}} \quad (4)$$

I_c is the moment of inertia of the crustal lattice plus whatever other stellar material is rigidly coupled to it, N_{ext} is the external torque acting on the star and N_{int} describes the coupling of the crust to those superfluid neutrons that couple to it on observable timescales. In the two-component model of Baym *et al.*²⁵, the internal torque is ascribed to the core neutron superfluid of inertial moment I_s and angular velocity $\Omega(t)$, and is assumed to take the form

$$N_{\text{int}} \equiv I_s \dot{\Omega} = (I_c/I) (\Omega_c - \Omega)/\tau_c \quad (5)$$

characteristic of simple mutual friction between the crust and core (where I is the total moment of inertia and τ_c is a crust-core coupling time). After a sudden initial jump $(\Delta\Omega_c)_0$ in Ω_c , the post-glitch behaviour described by equations (4) and (5) is

$$\Omega_c(t) = \Omega_0(t) + (\Delta\Omega_c)_0 [Qe^{-t/\tau} + (1-Q)] \quad (6)$$

where $\Omega_0(t)$ is the extrapolated frequency in the absence of the glitch, $\tau = \tau_c(I_s/I)$ and $Q \approx I_s/I$. The two-component theory provided a good fit to the initial post-glitch data from the Crab and Vela pulsars. For both pulsars, however, the Q and τ values required to obtain a fit differed somewhat from one glitch to the next. Moreover, Boynton and Deeter³⁷ showed that one could not fit the noise in the timing data on the Crab pulsar using those Q and τ values which had provided a satisfactory explanation of the larger Crab glitches, while data accumulation and the detailed analysis by Downs³⁰ of the post-glitch behaviour of the Vela pulsar following its four glitches, and by Lohsen²⁸ and Demianski and Proszynski³⁸ of the Crab pulsar following its two major glitches, provided a wealth of new data for which the simple two-component model could not provide an adequate explanation.

The physical origin of the giant glitches in the Vela pulsar posed an even greater puzzle: starquake theory⁹, although plausible for the Crab pulsar glitches³⁹, could not explain the magnitude and frequency of the Vela glitches. The most promising alternative which emerged was the idea of a sudden catastrophic unpinning of the pinned vortex lines in the crustal neutron superfluid every few years as a result of pulsar spin-down^{40,41}. In the course of exploring the behaviour of the pinned superfluid

between giant glitches, it was concluded that not only could the pinned superfluid be responsible for the giant glitches, but that the entire dynamics of post-glitch behaviour was probably related to the pinned superfluid; in other words, the post-glitch behaviour was the response of the pinned crustal neutron superfluid to a glitch, and physical processes must exist which couple the core neutron superfluid rapidly (on timescales $\leq$ days) to the crust^{18,42}. Thus, a general theory of the coupling between the pinned superfluid and the crust, which occurs by vortex creep (the motion of the pinned vortex lines in response to changes in the crustal rotation frequency⁴³), was developed. A detailed re-examination of the coupling between the crust and the core neutron superfluid was carried out^{26,27} in which additional physical mechanisms which couple the core neutrons rapidly to the crust were discovered, while the vortex creep theory was found not only to provide an excellent fit^{19,44} to the timing data^{28-30,38}, but to offer the possibility of determining the internal temperature of pulsars.

Neutron star coupling

Sauls, Stein and Serene²⁶ considered the structure of the vortices in the $^3P_2$ pairing state in the neutron superfluid core. They found that because of the small spontaneous magnetization that appears in the region near the vortex core, the coupling is qualitatively different from the relaxation mechanism considered by Feibelman⁴⁵, who calculated the scattering of electrons by magnetic moments of neutrons in low-lying excited states in the core of the vortex line. This requires thermal excitations and yields a relaxation time which is $\sim \exp(\Delta^2/k_B T E_F)$ (where k_B is Boltzmann's constant), whereas the electron magnetic vortex relaxation time is comparatively insensitive to the gap and temperature, going as the inverse power of $\Delta(T)$; the latter process gives relaxation times of about a year.

Alpar, Langer and Sauls²⁷ have examined the change in the structure of a neutron vortex line that results from the interaction between the neutron and proton condensates in the quantum liquid interior. Superfluid drag induces a proton charge current around each neutron vortex giving rise to a magnetic field confined within a screening radius ~ 50 fm, but of magnitude $\sim 10^{15}$ G, far larger than the spontaneous magnetization discussed above. The resulting charge-vortex line relaxation time is also insensitive to temperature and is $\tau_v \approx 20 P(s)$, where P is the rotation period. The dynamical coupling time of the core superfluid is

$$\tau \approx \frac{\tau_v}{x} \approx 400 P(s) \quad (7)$$

where $x \approx 0.05$ is the charged fraction of the neutron star core. This work provides crucial support for invoking only the crust superfluid in a model of pulsar glitches and post-glitch relaxation, which we now consider.

Vortex creep theory

The coupling between the pinned crustal superfluid and the crust is achieved by thermal creep of the vortex lines against pinning energy barriers, by analogy with flux creep in type II superconductors^{46,47}. In the steady state the pinned superfluid is coupled to the rest of the star and shares the effect of external torques. A glitch perturbs vortex creep away from its steady-state rate. Part of the pinned superfluid is thereby decoupled, and its gradual recoupling leads to observable behaviour. The sudden change in $\dot{\Omega}_c$ which accompanies the glitch is associated with sudden decoupling of the pinned superfluid region with an effective moment of inertia $\bar{I}_p$,

$$\bar{I}_p/I \equiv (\Delta\dot{\Omega}_c/\dot{\Omega}_c)_0 \quad (8)$$

and conservation of angular momentum gives

$$\bar{I}_p \delta\bar{\Omega}_p = I_c \Delta\Omega_c \quad (9)$$

where $\delta\bar{\Omega}_p$ is the mean decrease of the superfluid rotation rate and $\Delta\Omega_c$ is the magnitude of the glitch.

The angular velocity of the neutron superfluid is determined by the distribution of the pinned vortex lines. With azimuthal symmetry, the value of $\Omega(r) \equiv v(r)/r$ (where r is the cylindrical distance from the axis of rotation) is determined by the usual superfluid equations relating the macroscopic rotation rate to the distribution of quantized vortices:

$$\int_0^r (\nabla \times \mathbf{v}) \cdot d\mathbf{S} = \oint \mathbf{v} \cdot d\mathbf{l} = 2\pi\Omega(r)r^2$$

$$= \kappa \int_0^r 2\pi r' n(r') dr' \quad (10)$$

while vortex conservation leads to

$$\dot{\Omega} = -\kappa \frac{nv_r}{r} \quad (11)$$

Here, $n(r)$ is the number of vortices per unit area, $\mathbf{v}$ is the superfluid velocity, and v_r is the radial velocity of the vortex lines. From these equations it follows that $\Omega(r)$ can change only if vortices move. Their motion is described by an internal torque,

$$N_{\text{int}} \equiv \int_p dI_p \dot{\Omega}(r, t) \quad (12)$$

while the rate at which thermally activated vortex lines are able to tunnel between adjacent pinning configurations is

$$v_r = v_0 \exp \left[-\frac{E_p (\omega_{\text{cr}} - \omega)}{k_B T \omega_{\text{cr}}} \right] \quad (13)$$

where T is the crustal temperature, $\omega \equiv \Omega - \Omega_c$ is the superfluid angular velocity relative to that of the crust, and

$$\omega_{\text{cr}} \equiv (\Omega - \Omega_c)_{\text{max}} = \frac{f_p}{\rho \kappa r} \equiv \frac{E_p(\rho)}{\rho \kappa r \xi b} \quad (14)$$

is the maximum superfluid lag which can be supported by the pinning force, defined in equation (3). The approach to steady state is characterized by a relaxation time, τ_i , proportional to the temperature⁴³,

$$\tau_i = \left(\frac{\omega_{\text{cr}}}{E_p} \right) \frac{k_B T}{|\dot{\Omega}_{\infty}|} \equiv \frac{\alpha_i T}{|\dot{\Omega}_{\infty}|} \quad (15)$$

where $\dot{\Omega}_{\infty}$ is the steady-state value of $\dot{\Omega}_c$ and $\dot{\Omega}_i$, and the subscript 'i' refers to the pinning layer in question.

Glitches are catastrophic unpinning events extraneous to the creep process which itself involves a continuous slow vortex motion. The internal torque produced by the neutron superfluid in response to a glitch, $(\Delta\Omega_c)_0$, takes the simple form

$$N_{\text{int}}(t) = \sum_i I_i \dot{\Omega}_{\infty} \frac{1}{1 + (e^{t_{oi}/\tau_i} - 1)e^{-t/\tau_i}} \quad (16)$$

where the I_i are the moments of inertia of the distinct pinning layers; the t_{oi} which appear in equation (16) are post-glitch delay times. For pinning layers where there is no glitch-associated vortex motion,

$$t_{oi} = \frac{(\Delta\Omega_c)_0}{|\dot{\Omega}_{\infty}|} \quad (17)$$

and in those regions where there is glitch-associated vortex motion,

$$t_{oi} = \frac{\delta\Omega_i}{|\dot{\Omega}_{\infty}|} = \frac{X_i \kappa}{2\pi r^2 |\dot{\Omega}_{\infty}|} \quad (18)$$

where $\delta\Omega_i$ is the glitch-associated change in the superfluid rotation frequency and X_i is the number of vortices that have moved through the region.

The vortex creep theory of post-glitch response differs from the two-component model²⁵. (1) It involves a number of distinct components which, however, make up only a comparatively small fraction (~a few per cent) of the neutron superfluid, namely the pinned superfluid in the crust. This is borne out by the observed values of $(\Delta\dot{\Omega}_c/\dot{\Omega}_c)_0 \sim 10^{-3}$ – 10^{-2} (compare with

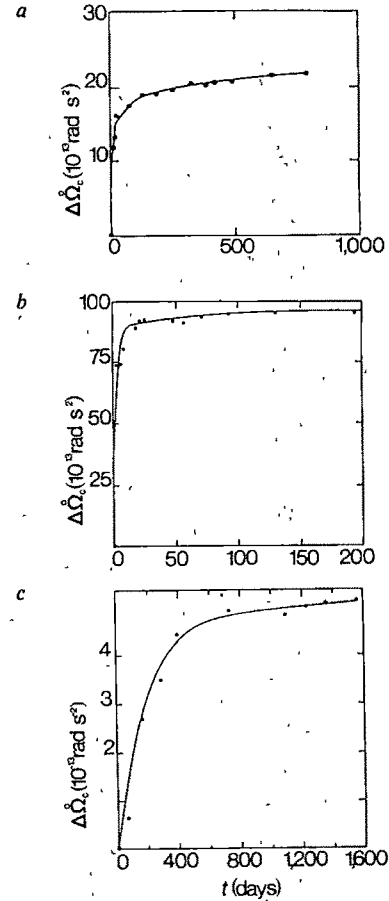


Fig. 3 Fits to the post-glitch $\dot{\Omega}_c(t)$. a, Representative post-glitch fit for the Vela pulsar¹⁹; $\dot{\Omega}_c(t)$ following the glitch of July 1978. b, Fit to $\dot{\Omega}_c(t)$ following 1975 Crab pulsar glitch⁴⁴. c, Fit to $\dot{\Omega}_c(t)$ following the glitch observed in PSR0525+21 (ref. 44). In each case, $t=0$ denotes the epoch of the first post-glitch timing observation and $\Delta\dot{\Omega}_c(t) \equiv \dot{\Omega}_c(t) - \dot{\Omega}_c(0)$.

equation (8)). (2) It is a fundamentally nonlinear theory of superfluid response; glitch-associated vortex creep depends exponentially on the size of the glitch (in regions in which no vortex motion occurs) and on the change in the superfluid velocity (in regions in which vortex motion occurs during the glitch). (3) It leads to relaxation times proportional to the internal temperature of the star, so that timing observations can provide a unique probe of the internal stellar temperature. This internal temperature can then be used to obtain an estimate of the surface temperature.

Thermally activated vortex creep leads to an internal dissipation of the stored rotational energy of the neutron star. The magnitude of the continuous energy dissipation resulting from creep follows directly from the superfluid equations of motion, and is

$$\dot{E}_{\text{diss}} \equiv |\dot{\Omega}_c| \int_p dI_p \omega_{\infty} \equiv |\dot{\Omega}_c| I_p \bar{\omega}_{\infty} \quad (19)$$

where I_p is the moment of inertia of the entire set of pinning layers, $\bar{\omega}_{\infty}$ is an average through the pinning layers and ω_{∞} is the steady-state value of $\Omega - \Omega_c$. This dissipation is the principal source of heat for old pulsars which have radiated away their original heat content.

Vela pulsar glitches

Alpar *et al.*¹⁹ have shown that the observed complex post-glitch behaviour of the Vela pulsar following the four glitches which took place in the decade 1969–79 (ref. 30) finds a straightforward explanation in terms of vortex creep theory. They fit the observational data of Downs with the following expression for $\dot{\Omega}_c(t)$,

$$I_c \dot{\Omega}_c(t) = N_{\text{ext}} - N_1 - N_2 - N_A \quad (20)$$

Here N_1 and N_2 are specified by equations (16) and (17), with relaxation times τ_1 and τ_2 . These torques represent an average response of two distinct pinning layers through which no vortices move. A third region, through which vortices move, is represented to a first approximation by

$$N_A \approx \dot{\Omega}_\infty \frac{I_A t}{t_{OB}} \quad (21)$$

(ref. 19), where I_A is the moment of inertia of the superfluid in pinning layers (where vortices unpin and repin) and t_{OB} is the post-glitch delay time given by equation (18) on taking X_1 to be the total number of vortices that unpin. A choice of $\tau_1 = 3$ days and $\tau_2 = 60$ days, for each of the four glitches, gives an excellent fit to the observational data (Fig. 3). Alpar *et al.*¹⁹ conclude that the two distinct pinning layers correspond to weak pinning and superweak pinning respectively, characterized by $\langle \alpha^{sw} \rangle \sim 2 \times 10^{-17}$ days $K^{-1} s^{-2}$ and $\langle \alpha^w \rangle \sim 4 \times 10^{-16}$ days $K^{-1} s^{-2}$ (see equation (15)). Within each pinning region the actual pinning parameters may vary either way by factors of ~ 2 . The size of the inertial moments of these pinning regions ($\sim 10^{-2} I$) is physically reasonable, in terms of current neutron-star models.

From the observed relaxation times, Alpar *et al.*¹⁹ estimate the internal temperature of the Vela pulsar to be $(1.5 \pm 1) \times 10^7$ K. The quoted errors do not refer to a particular calculation, but rather reflect an estimate of the degree of uncertainty introduced by lack of knowledge of various pinning parameters.

Crab pulsar

Alpar, Nandkumar and Pines find, surprisingly, that the post-glitch behaviour of the Crab pulsar following its two large glitches in 1969 and 1975 could be fitted (see Fig. 3b) with the extreme (Occam's razor) hypothesis that both the internal structure and the vortex pinning sites in the Crab pulsar are essentially the same as those hypothesized for the Vela pulsar, so that the only significant physical differences between the two pulsars reside in the spin down rate and internal temperature⁴⁴. In this fashion they deduce an internal temperature for the Crab pulsar of 3.8×10^8 K, with, again, an uncertainty either way of a factor of ~ 2 . The surface temperatures of the Vela and Crab pulsars can be estimated using the scaling laws obtained by Gudmundsson *et al.*⁴⁸. For a $1.4 M_\odot$ neutron star with a reasonably stiff equation of state, one finds the surface temperatures observed at infinity are $T_{\infty, \text{Vela}} \approx 3 \times 10^5$ K and $T_{\infty, \text{Crab}} \approx 1.6 \times 10^6$ K. For both pulsars, these surface temperatures are well below the upper limits obtained by the Einstein observatory^{49,50}. Comparing our estimates with calculations on neutron star cooling and evolution (see ref. 51 for recent review), one finds that an internal temperature ratio of 25 is not consistent with the standard cooling calculations, which, for a given Crab temperature would predict a considerably hotter Vela pulsar. Therefore, these results may provide the first indication that non-standard, more rapid cooling scenarios (perhaps reflecting the presence of a pion condensate, if a pion condensate indeed leads to sufficiently rapid cooling) are in operation in neutron stars.

Old pulsars

Consider now pulsars which are old enough to have radiated away their original heat content so that their dominant source of heat is due to internal dissipation by vortex creep. On equating the rate of dissipation $\dot{E}_{\text{diss}}$ (equation (19)) to the stellar luminosity, one finds a limiting black-body surface temperature of

$$T_s \approx 1.1 \times 10^5 [I_{p,43} \bar{\omega}_{cr} \Omega_{-14} / R_6^2]^{1/4} \quad (22)$$

From the upper limit to the surface luminosity of the Vela pulsar in the soft X-ray region⁴⁹ and the Einstein detection of PSR1929+10 (ref. 52), we have estimated $I_{p,43} \bar{\omega}_{cr}$ to be ≤ 1 (ref. 53). For such a pulsar, the thermal flux given by equation (22) is small enough for the internal temperature to be close to the surface temperature. If all old pulsars possess essentially the same pinning layers as the Crab and Vela pulsars, they would have temperatures determined by internal dissipation according

to equation (22) and would possess creep relaxation times that depend only on their spin-down rate, and are

$$\tau_{sw}^{\text{old}} \sim 220 |\Omega_{-14}|^{-3/4} \text{ days} \quad (23)$$

$$\tau_w^{\text{old}} \sim 4.4 \times 10^3 |\dot{\Omega}_{-14}|^{-3/4} \text{ days} \quad (24)$$

for the superweak and weak pinning layers, respectively.

Among the old pulsars which have exhibited glitches, post-glitch timing observations are available only for PSR0525+21. Alpar, Nandkumar and Pines⁴⁴ (Fig. 3c) find that vortex creep theory provides a good fit to the observed post-glitch behaviour of this pulsar with a 'fast' relaxation time of ~ 150 days and is consistent with the additional presence of a much slower 3,000-day relaxation process. The vortex creep theory prediction of τ for PSR0525+21 is, from equation (24), some 140 days, in excellent agreement with this fit. Thus vortex creep theory can explain the post-glitch behaviour of both very young and very old pulsars. What is striking is that it does so without involving new parameters for each glitch or pulsar.

Superfluidity in millisecond pulsars

The recently discovered millisecond pulsars^{54,55} have much larger angular velocities, Ω , and much lower inferred dipolar fields, B_s , than 'ordinary' pulsars. Neither of these changes alters the superfluid physics of the neutron star⁵³. The search for observational consequences of superfluidity in millisecond pulsars thus becomes a search for pinned crustal superfluid phenomena: (1) the internal generation of heat by vortex creep; (2) macroglitches, such as those observed in the Vela pulsar, produced by the simultaneous unpinning of a large group of vortex lines; (3) post-glitch timing behaviour associated with the gradual recoupling of the pinned superfluid; (4) timing noise.

The first three phenomena scale with $\dot{\Omega}_c$, whereas the value of $|\dot{\Omega}_c|$ ($= 2.9 \times 10^{-13}$ rad s^{-2}) for the millisecond pulsar PSR1937+21 resembles that of ordinary pulsars. Our results are not encouraging for would-be observers of glitches in millisecond pulsars; although our estimated glitch intervals ($\sim 10^2$ to $\sim 10^4$ yr) are comparable with those found in the conventional pulsar sample³⁵, the present sample of millisecond pulsars is too small to make it likely that a substantial glitch in one of these will be observed in the near future.

The timing from millisecond pulsars is of great importance in view of the potential practical applications of the excellent timing accuracy⁵⁶ of PSR1937+21. To estimate the contribution of the unpinning events to timing residuals, we take the rate of unpinning events to be $R \sim |\dot{\Omega}_c| / \delta \Omega_{cr}$ where $\delta \Omega_{cr}$ is a critical lag between superfluid and crust rotation rates needed to trigger a typical unpinning event, and assume that the unpinning event involves an angular momentum transfer of order $\Delta L = I_c \Delta \Omega_c \approx I_s \delta \Omega_{cr}$ where I_s is the moment of inertia of the region through which vortices move in a typical unpinning event. The noise strength S then scales with $\dot{\Omega}_c$:

$$S \equiv R \langle \Delta \Omega_c^2 \rangle \sim |\dot{\Omega}_c| \left(\frac{I}{I_s} \right)^2 \delta \Omega_{cr} \quad (25)$$

There is some evidence⁵⁷ for a correlation of S with $|\dot{\Omega}_c|$. On scaling from the observations of white $\dot{\Omega}_c$ noise in the noisiest source, the Crab pulsar, and expressing the result as an r.m.s. timing residual⁵⁸, one has

$$\langle R^2 \rangle_{1937+21, \text{intrinsic}}^{1/2} \sim 4 \times 10^{-2} \mu s \quad (26)$$

$\sim 5\%$ of the current residual $\approx 0.9 \mu s$ (ref. 56). Thus, one can be (cautiously) optimistic that the intrinsic contributions to the noise in PSR1937+21 are quite small. Note, however, that if the events are triggered by a critical value $(\delta \Omega / \Omega)_{cr}$ instead of $\delta \Omega_{cr}$, $S \propto \dot{\Omega}_c \Omega_c$ and scaling from the Crab pulsar yields an estimate of intrinsic noise comparable with the observed residuals in PSR1937+21.

Accreting X-ray sources

We have concentrated on effects of superfluidity in radio pulsars, where the external torque can be taken to be constant on time-

scales of the observed variations in timing behaviour. In the new picture of neutron-star superfluids that we have presented, only $\sim 10^{-2}$ of the superfluid interior, the pinned superfluid in the crust, produces observable deviations from rigid-body behaviour, and the resulting Ω_c variations are of the same order. Such variations are clearly unimportant in comparison with the observed variations in the accretion torque in systems like Her X-1 or Vela X-1 (ref. 59 and F. K. Lamb *et al.*, in preparation), so that the superfluid interior is negligible as a source of timing noise in accreting X-ray pulsars.

The dynamics of the pinned superfluid does, however, place severe constraints on precession (wobble) frequencies and damping times, which in effect rule out neutron-star precession as the explanation of various quasi-regular timescales observed in X-ray binaries (for example, the 35-day cycle in Her X-1). First, Shaham⁶⁰ has pointed out that the presence of pinned superfluid leads to wobble frequencies Ω_w given by $\Omega_w/\Omega \sim I_p/I \sim 10^{-2}$, where Ω is the rotation frequency and I the total moment of inertia. Second, precession of the crust will be damped by its coupling to a fluid interior. The damping time is $\tau_d \equiv \tau\Omega/\Omega_w$ (ref. 61) where τ is the dynamical coupling time, and is thus of the order of 5 days for a neutron star with a 10-s rotation period.

Concluding remarks

We have described in this article the key role which synoptic observations and detailed analysis of the timing data of individual pulsars has played in bringing about a substantive change in perspective on post-glitch relaxation and the astrophysical evidence for neutron superfluidity. Without observational data of this quality there would have been little incentive to

develop a detailed theory of pinned vortex motion. Future careful observations of Crab, Vela and other pulsars will be of assistance in pointing out directions in which the theory can be improved and refined.

Much work has been done on neutron-star superfluidity which, unfortunately, there has not been space to discuss here. This includes work on the nature of pion condensates in neutron stars^{11,12,62,63}, neutron-star cooling⁵¹, the dynamics of moving vortex lines⁶⁴, as well as work in progress (M.A.A., R. Nandkumar and D.P., in preparation) on information concerning vortex creep theory which may be provided by the recent analysis (ref. 65 and P. Boynton and J. Deeter, in preparation) of timing noise observations to 23 pulsars in addition to Crab and Vela.

It could be argued that, although a convincing case has been made for crustal neutron superfluidity, there is little observational evidence for core neutron superfluidity, as the calculated rapid coupling of the core superfluid to the crust rules out post-glitch behaviour as a way to observe core superfluids. Better observations of pulsar surface temperatures and improved theories of neutron-star cooling may offer the best prospects for determining the role played by core superfluids. As we have noted, rapid cooling of pulsars during the epoch from $\sim 10^3$ to 10^4 yr is required by the internal temperatures inferred above for the Crab and Vela pulsars. This may provide a cogent theoretical and observational argument in favour of pion condensation, but more work will need to be done before any definitive conclusions can be reached.

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Punctiform initiation of seafloor spreading in the Red Sea during transition from a continental to an oceanic rift

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Transition from a continental to an oceanic rift occurs in the Red Sea by initial emplacement of oceanic crust in regularly spaced 'hot points', which serve as nuclei for axial propagation into segments of oceanic crust accretion and for initiation of seafloor spreading. Hot points develop above upwelling mantle diapirs caused by density/viscosity inversion beneath continental and oceanic rifts.

AN important goal in earth science is to understand how the transition from a continental to an oceanic rift occurs in time and space, which will further our knowledge of oceanic Atlantic-type passive margins. This transition, which occurred along the proto-Atlantic rift 160–90 Myr ago, can be observed today in the northern Red Sea. Based on morphotectonic, magnetometric, seismic-reflection and petrological data from this region, I will attempt to demonstrate that: (1) injection of oceanic crust takes place initially in punctiform hot areas axially spaced ~50 km apart; (2) activation of hot points shows a northward time progression; and (3) each hot point serves as a nucleus for axial propagation of the zone of oceanic crust accretion into linear segments of spreading. I will also explain the punctiform initiation of seafloor spreading in terms of inferred patterns of upper mantle upwelling beneath the Red Sea, and suggest that the same model can be applied to the initial opening of the Atlantic rift.

Plate-tectonic reconstructions of the Red Sea/Gulf of Aden region^{1–5} suggest that, although emplacement of oceanic crust between Arabia and Africa initiated in the Gulf of Aden as early as ~10 Myr ago⁶, it started more recently in the Red Sea⁷. An axial rift valley with strong magnetic signature is present almost continuously in the southern Red Sea between 16° and 19° N. Vine–Matthews-type axial magnetic anomalies indicate initiation of seafloor spreading by injection of basalt crust in a narrow axial zone ~5 Myr BP in a region around 17° N, but more recently to the north and south of this region⁸, in a pattern which suggests axial rift propagation⁹. Moving northward, the axial rift valley and associated magnetic signature become discontinuous and then disappear completely in the northern Red Sea (Figs 1, 2).

This description assumes that, outside the axial zone with clear-cut high-amplitude Vine–Matthews magnetic anomalies, the Red Sea is floored by thinned, stretched continental crust criss-crossed by diffuse basaltic dyke injections, a view supported by recent geophysical data collected at sea^{7,10–12} and by observations at Zabargad (St John) Island, an uplifted block of Red Sea lithosphere¹³. However, the concept that the whole width of the Red Sea formed by two stages of seafloor spreading, the first of which started as early as ~40 Myr BP, has also been proposed^{14,15}.

The axial zone of the Red Sea can be divided from south to north into the following regions (Fig. 1), based on their different morphotectonic and magnetometric characteristics^{7,8,12,16–18}: (1) rift valley region (17° N–19°30' N), with a relatively straight and continuous axial rift valley and linear magnetic anomalies; (2) multi-deeps region (19°30' N–22° N), with a complex pattern of axial deeps and troughs; (3) transitional region (22° N–24° N), with a few regularly spaced axial trough segments; (4) northern region (north of 24° N), with no axial rift valleys but a few

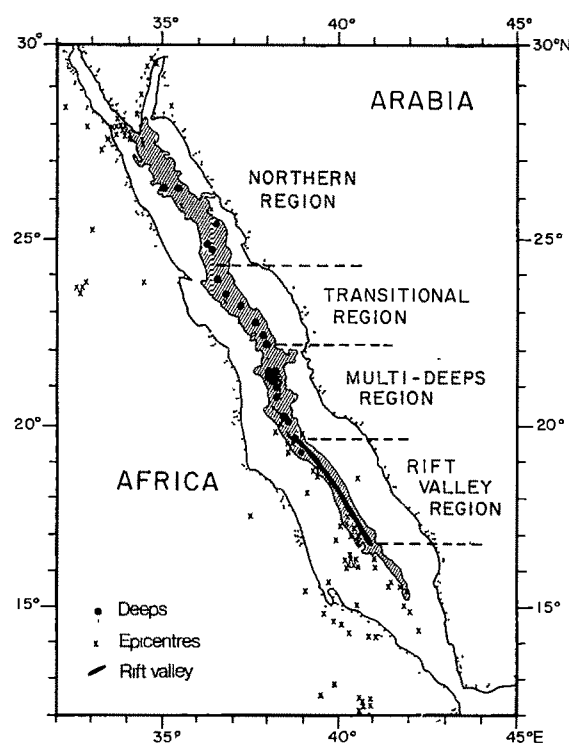


Fig. 1 Outline of the Red Sea, with the axial zone enclosed by the 500-fathoms isobath. The approximate location of the axial rift valley and of isolated deeps and troughs is indicated. Also shown are locations of earthquake epicentres of magnitude >4 recorded from 1953 to 1983. The axial zone has been subdivided into geotectonic regions (see text)

isolated deeps. The model proposed here is based primarily on results obtained from the transitional region.

Red Sea transitional region

Field work in this region (Fig. 2) has shown the existence of segments of axial troughs and deeps with high-amplitude magnetic anomalies, separated by intertrough zones which lack an axial rift valley and high-amplitude magnetic anomalies, and have continuous, thick sediment cover across the axis^{11,16–18}.

The transitional region axial trough segments, named (from south to north) Thetis, Nereus, Bannock and Vema^{11,16}, become shorter, narrower and shallower moving from south to north (Fig. 2a, b). The Thetis and Nereus segments display morphotectonic features typical of rift valleys of oceanic slow-spreading axial zones such as the Mid-Atlantic Ridge (MAR). They have

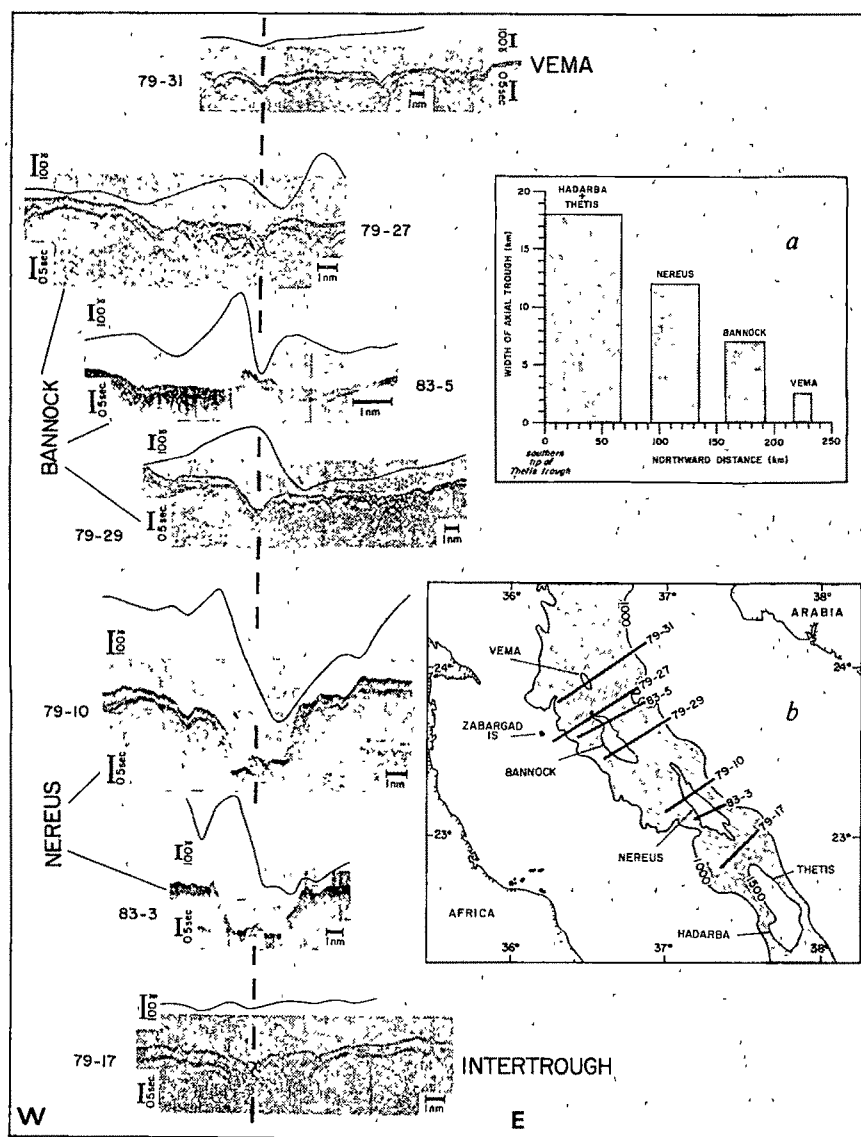


Fig. 2 Outline of the Red Sea transitional region where the axial troughs and deeps (Thetis-Hadarba, Nereus, Bannock and Vema) are shown enclosed by the 1,500-m isobath (insert *a*). A series of seismic reflection profiles taken across the Red Sea axis (locations indicated in insert *b*) is shown on the left of the figure, together with magnetic anomalies along the same profiles. The vertical broken line indicates the approximate axis of the Red Sea. Insert *b* shows the width and length of the Thetis-Hadarba, Nereus, Bannock and Vema axial trough and deeps, defined by the 1,500-m isobath. Length is estimated from the distance along the Red Sea axis taken from the southern end of the Thetis-Hadarba trough.

steep walls formed by inward-facing normal faults¹⁸ and a neovolcanic zone floored by young basalts with mid-ocean ridge basalt (MORB) affinity¹¹. A trough segment to the south of Thetis, termed Hadarba Deep¹⁶, merges with Thetis although the axes of the two segments are offset laterally by ~5 km (Fig. 2*b*). High-amplitude magnetic anomalies are present at Thetis¹⁵, although its scant coverage does not allow an accurate estimate of the time of initiation of seafloor spreading. Its wider, longer and deeper trough suggests that Thetis is older and more evolved than Nereus. Magnetic anomalies over the Nereus trough suggest the presence of oceanic crust and initiation of seafloor spreading ~1 Myr ago in sections across the northern and southern parts of the trough, but >2 Myr ago in sections from the central part of the trough¹¹. Thus, the Nereus axial trough could be considered a mini-propagating oceanic rift segment where oceanic crust was emplaced initially in a central spot which then served as a nucleus for axial propagation.

The next axial deep segment to the north of Nereus, the Bannock Deep, lacks a steep axial rift valley but shows a gentle graben-like morphology with continuous sediment cover except along one section where a 500-m-high basement protrudes from the sediment (Fig. 2, profile 83-5). This feature is probably a volcanic seamount because fragments of dolerite were recovered from it¹¹. Profiles taken across the axis a few kilometres north and south of the seamount indicate continuous sediment cover and a prominent fault aligned axially with the volcanic seamount (Fig. 2). Narrow high-amplitude magnetic anomalies are associated with the seamount and the axial fault.

The Vema axial deep consists of a relatively gentle depression with continuous sediment cover and no high-amplitude magnetic anomalies, but there is relatively high heat flow¹¹. A consistent pattern of axial high-amplitude magnetic anomalies is absent in the Red Sea north of the Vema Deep, although deeps and anomalies have been found at several isolated sites^{16,18,19}.

Punctiform initiation of seafloor spreading

The observations from the Red Sea transitional region lead to the following interpretations, summarized in part in Fig. 3:

(1) The initial breakthrough of oceanic crust and the beginning of organized seafloor spreading occurs in essentially punctiform hot areas located in axially oriented segments of tensional fissures. The initial breakthrough of oceanic crust would occur through continental crust which has been thinned, stretched and diffusely injected by basaltic dykes^{7,10,11}, although it could occur in ancient, cold oceanic crust if the two-stage opening model^{14,15} is accepted. The igneous body cropping out from the axis of the Bannock graben and the axial fault detected just south and north of this igneous body are examples of recent hot point formation.

(2) Axial propagation from each hot point gives rise to linear spreading segments⁹. This hypothesis is supported by the detection of magnetic anomalies, as old as 2-3 Myr in sections from the centre of the Nereus trough segment, but younger close to the tips of the segment.

(3) A northward progression can be detected in the time of initial activation of the punctiform areas of ocean crust emplace-

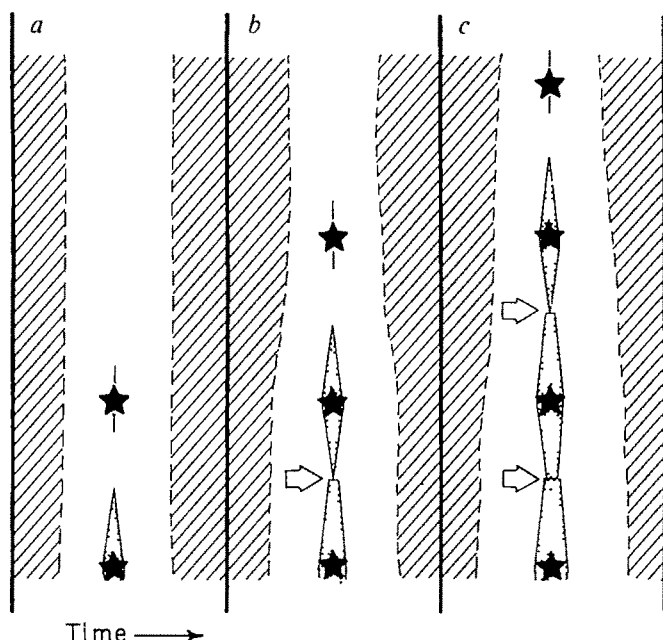


Fig. 3 Schematic model of the initiation of seafloor spreading in the Red Sea transitional region. *a-c*, Different stages in time. Stars indicate sites of hot points where the initial emplacement of oceanic crust occurs. Striped areas, continental; stippled areas, oceanic; open areas, intermediate continental crust stretched, thinned and injected by basaltic dykes. Arrows, discontinuities in the morphotectonic and magnetic axial pattern, predicted by the model.

ments. Although in the southern Red Sea (between 17° N and 18° N), seafloor spreading initiated ~5 Myr BP, emplacement of oceanic crust probably started between 2 and 3 Myr BP at Nereus; it started <1 Myr BP at Bannock and has not yet started at Vema. The observation that the Thetis trough is deeper and wider than Nereus suggests that it is more evolved, and is therefore older than Nereus. Relatively high heat flow, as detected within the Vema Deep¹¹, may be a precursor to oceanic crust emplacement and may therefore mark future hot points in the northern Red Sea.

(4) The distance between the centre points of Thetis, Nereus, Bannock and Vema is ~50 km (Fig. 2*b*), suggesting a regular spacing of the areas of upper mantle upwelling and oceanic crust initial emplacement.

(5) Axial propagation from each hot point will eventually lead to joining of the different rift segments. Discontinuities are likely to develop in the axial morphotectonic and magnetic lineations where the tip of rift segments derived from different hot points join (Fig. 3). Moreover, the axial troughs and deeps are not all aligned; some show lateral offsets (Fig. 2). These initial offsets in the segments of oceanic crust emplacement may or may not lead to the formation of initial transform faults.

(6) The regular segmentation of the axis of spreading (Fig. 3) is interrupted where the Red Sea lithosphere is intersected by pre-existing shear zones²⁰, which would prevent normal axial rift propagation and act as 'locked zones' in the sense described by Courtillot⁹.

I now consider how the proposed model, derived from observations in the transitional region, fits available data from other regions of the Red Sea.

Rift Valley region. The evolution of seafloor spreading from a punctiform initiation can be inferred from observations obtained in the southern Red Sea between 17° N and 19°30' N (Fig. 1). The relatively continuous axial trough and the associated high-amplitude magnetic anomalies imply a steady though asymmetrical seafloor spreading regime for the past few million years, with spreading being about two-thirds faster to the west than to the east^{8,16}.

A detailed investigation of the axial zone at 18° N, including direct submersible observations^{21,22}, has revealed a morpho-

tectonic structure of the axial trough similar to that of the MAR in the FAMOUS area, a neovolcanic zone with basalts similar to MORB, and a spreading rate averaging 1.6 cm yr⁻¹ for the past 3-5 Myr. A deep-tow profile (Fig. 4) of the axial zone at 17°30' N (ref. 23) confirmed a morphotectonic structure similar to that found in the FAMOUS area, and demonstrated asymmetrical spreading (≈ 5 mm yr⁻¹ to the east, 10 mm yr⁻¹ to the west) starting at 4.8 Myr BP. In the context of the proposed model it is of interest that the Rift Valley morphotectonic structures and high-amplitude magnetic anomalies run along the axis in this region for straight segments 30-50-km long, which are stepped laterally by small, 3-10-km transform or non-transform relay zones (Fig. 4) where the magnetic anomalies are weak and irregular¹². One such discontinuity was surveyed in detail at 18° N (ref. 21). The proposed model explains these discontinuities as marking sites where different rift segments meet, each created by longitudinal propagation from an initial hot point (Fig. 3).

Multi-deeps region. This region, located between the more evolved southern Red Sea and the transitional region (Fig. 1), displays a complex geotectonic situation: the straight axial rift valley is replaced by a network of deeps distributed partly in an 'echelon' pattern^{16-18,24}. Some of these deeps are thermally active at present and contain hot brines (for example, Atlantis II, Discovery), some are instead inactive²⁴. This complex geotectonic structure may be determined in part by the presence of a major transverse fracture zone intersecting the Red Sea axis^{17,20,25}, an interpretation supported by the clustering of recent earthquake epicentres in this area (Fig. 1), and by the orientation of magnetic anomalies transversal to the Red Sea axis¹⁷. This is one of three major fracture zones intersecting the Red Sea, the other two being the Zabargad and the Brothers fracture zones, all striking parallel to the Dead Sea-Aqaba fault²⁰.

Northern region. The Red Sea north of the Vema Deep lacks a regular pattern of axial rift valley segments and associated high-amplitude magnetic anomalies^{7,11}, though a few isolated deeps are present, some located off the axis (Fig. 1). Some are associated with magnetic anomalies; that is, Charcot Deep at 25° N, where non-MORB basalts intrude the sediment¹⁹, and Al Wajab Deep at 25°20' N, where the anomalies are lineated partly in a direction transversal to the Red Sea axis^{15,19}. Some of these deeps may represent small pull-apart basins caused by tectonics related to the fracture zone, such as Al Wajab, at the northeastern end of the Zabargad Fracture Zone, where the Red Sea axial direction shifts by $\approx 40^\circ$ (Fig. 1).

Following Cochran⁷, I interpret the northern Red Sea region as floored by thinned and stretched continental crust injected by diffuse basaltic intrusions. Probable examples of such intrusions are basaltic/gabbroic rocks from the Brothers Islets²⁶ and the Charcot Deep¹⁹, showing transitional or alkaline affinity, in contrast to the MORB-like characteristics of basalts from the Nereus Trough¹¹ and from other axial sites further south in the Red Sea²⁷. Thus, punctiform axial injection of oceanic crust and organized seafloor spreading have probably not yet started in the northern region of the Red Sea.

Mantle upwelling beneath the Red Sea

The punctiform model of initiation of seafloor spreading in the Red Sea implies that beneath each initial hot point there is a zone of preferential upwelling of upper mantle material. It is generally agreed that a broad thermal anomaly affects the upper mantle in major rift zones of the Earth. One of the tenets of plate-tectonic theory is that the asthenosphere/lithosphere boundary rises close to the surface along the axis of oceanic rifts²⁸. A rise of the upper-mantle isotherms and of the asthenosphere/lithosphere boundary has been documented for major continental rifts, such as the East African²⁹, Rio Grande^{30,31}, Rhinegraben³² and Baikal^{33,34} rifts. A rise of upper-mantle isotherms and a thinning and stretching of the lithosphere, probably resulting in part from convective heat transfer to its base³⁵, obtains also beneath the Red Sea rift, a view consistent

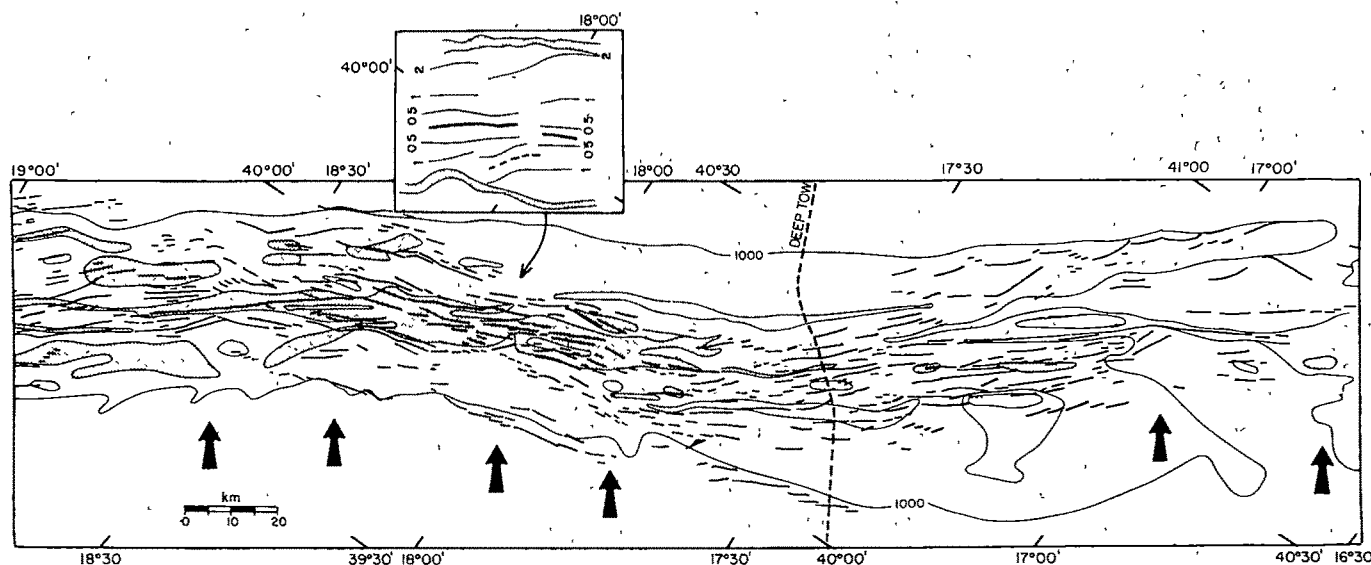


Fig. 4 A portion of the axial zone of the rift valley region of the Red Sea, with simplified bathymetry, from Backer *et al.*¹⁶, and structural lineations obtained by a GLORIA sidescan survey, from Garfunkel *et al.*¹². Arrows indicate discontinuities in the axial morphotectonic lineations observed by Garfunkel *et al.*¹². Insert shows details of one such discontinuity at 18°, simplified from Zonenshajn *et al.*²¹. Isochrons (in Myr), based on magnetic anomalies, are indicated. Thick full line, present axis, dashed thick line, fossil axial segment; shadowed area, outer slopes of rift valley.

with a broad band of high heat flow³⁶ and positive gravity anomaly³⁷ extending across the entire width of the Red Sea.

Continuous upwelling and decompression of upper-mantle material below rift zones leads to partial melting. A low-density/viscosity layer containing a continuously replenished small melt fraction would result, sandwiched between material of higher density and viscosity. The low density of this layer is enhanced by the probability that the upwelling mantle is injected by metasomatic, H₂O- and CO₂-rich fluids, resulting in H₂O-containing phases, such as amphiboles. This mantle metasomatism is important below continental rifts³⁸.

The presence beneath the Red Sea of an upper mantle zone containing a melt fraction and affected by metasomatism is suggested by studies of a mantle-derived spinel lherzolite body uplifted on Zabargad Island (Fig. 2b), probably during the early stages of Red Sea rifting^{13,39}. The lherzolite body contains zones of plagioclase peridotite, probably representing small fractions of melt trapped by lherzolites in the upper mantle, as well as amphibole peridotites produced by reactions of metasomatic fluids with the lherzolite⁴⁰.

The density/viscosity inversion in the upper mantle can be treated as a Rayleigh-Taylor-type instability, an approach which has been used to model the distribution of volcanic activity along island arcs⁴¹ and oceanic spreading zones^{42,43}. Theory and experiments^{41,44-47} indicate that the interface between a high density/viscosity upper layer and a layer below with lower density/viscosity develops a sinusoidal instability that may evolve into a pattern of λ -spaced injections of the lower density fluid into the denser layer above. The density contrast affects the velocity of upwelling of the diapirs according to Stoke's law. Their spacing, λ , is related to the ratio of the thicknesses of the upper and lower layer, h_1/h_2 , and the ratios of their viscosities, μ_1/μ_2 (refs 45-47).

In the case of the Red Sea rift, the low-density upper mantle

zone can be assumed to be elongated in shape and of limited width. Provided $h_1 > h_2$, the spacing of the diapirs surging from the lower layer is approximated by^{41,45,46}:

$$\lambda = \frac{2\pi h_2}{2.15} (\mu_1/\mu_2)^{1/3}$$

Assuming μ_1 to be of the order of 10^{20} P^{48,49}, μ_2 can be estimated as being lower by 2-4 orders of magnitude, depending on the degree of partial melting chosen for h_2 and on the temperature and pressure contrast of the two layers. The thickness of h_2 depends on the upper mantle thermal regime of the particular rift being considered. Assuming h_2 as the layer containing melt within the 5-10% range, its thickness beneath the Red Sea axis should be a few kilometres, as estimated from data from Iceland⁵⁰ and mid-ocean ridges^{43,48}. If $\mu_1/\mu_2 = 10^3$ and $h_2 = 2$ km are chosen, a spacing slightly over 50 km is obtained for the upwelling diapirs.

Upwelling of the diapirs would result in an increased degree of melting, with the melt tending to segregate from its parent, collect towards the top of the diapirs and hence be injected into the crust. The attenuated thickness of the Red Sea crust would permit the 'asthenosphere geoid'^{51,52} and hence the melt to reach the sea floor. This mantle-driven pattern leading to the punctiform geometry of initial breakthrough of oceanic crust (Fig. 5), however, is interrupted where the Red Sea lithosphere is intersected and disrupted by pre-existing fracture zones, as in the multi-deeps region and in the Zabargad Fracture Zone.

The northward time progression in the activation of the hot points and in the evolution of seafloor spreading in the Red Sea suggests that the asthenospheric diapirs are superimposed on a broader zone of shallow hot upper mantle which may be moving as a wavefront towards the northern Red Sea (Fig. 5).

Axial propagation from each initial hot point could take place by mechanisms similar to the asthenospheric longitudinal flow

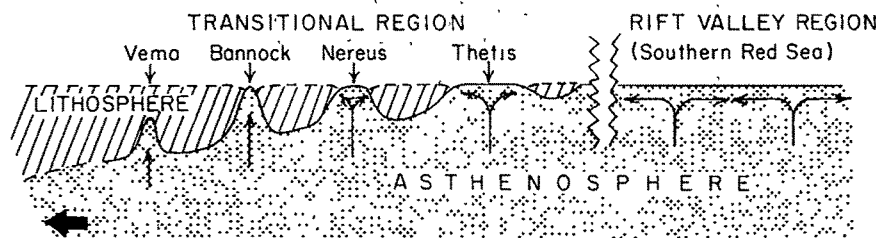


Fig. 5 Schematic and hypothetical cross-section along the axis of the Red Sea, showing regularly spaced asthenospheric diapirs in the transitional region, possibly caused by Rayleigh-Taylor instability in the upper mantle, superimposed on a broad wavefront of shallow hot mantle moving northward beneath the Red Sea.

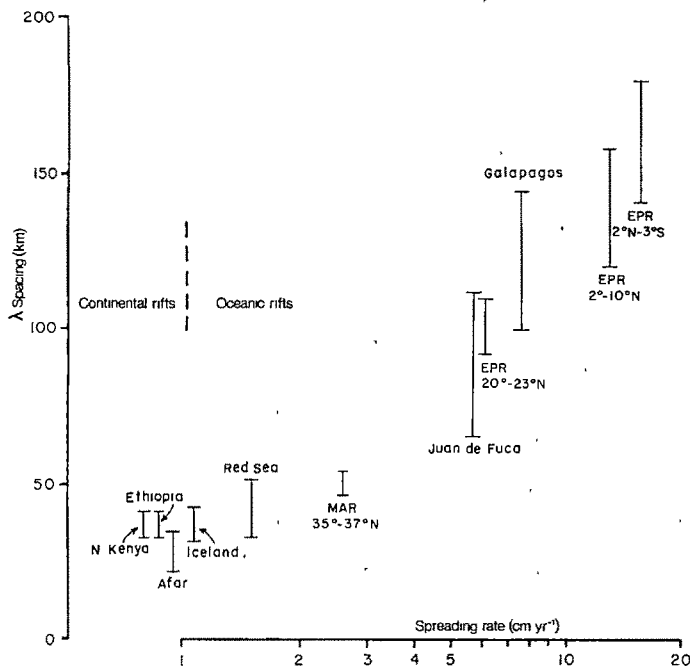


Fig. 6 Spacing of axial volcanism in continental, proto-oceanic and oceanic rifts, versus spreading rate for the oceanic rifts. Data for the East Pacific Rise (EPR), MAR, Galapagos rift and Juan De Fuca Ridge are from Crane⁴³. Data for the Red Sea are from the present paper, for Iceland they include Quaternary and Recent volcanism of the East Volcanic Zone, from Sigurdsson and Sparks⁵⁶; for North Kenya, Ethiopian and Afar rifts they include Quaternary and Recent volcanism, from Mohr and Wood⁵⁷.

described by Courtillot⁹ and Vogt⁵³, and would result in axial spreading segments similar to those 30–50-km long observed in the rift valley region of the southern Red Sea. Interestingly, Mesozoic (155–108 Myr BP) magnetic anomalies from the western North Atlantic reveal that close to the time of opening, the early Atlantic accretionary axis was divided in segments

each ~50 km long with discontinuities of the magnetic anomalies, not necessarily associated with transform offsets, between each segment⁵⁴. Therefore, it seems that the punctiform model of initiation of seafloor spreading proposed for the Red Sea can also be applied to the early Atlantic rift.

Evolution from continental to oceanic rift

Structural studies of peridotites from ophiolite complexes suggest vertical flow of upper-mantle material beneath oceanic ridges, with upwelling zones spaced 50–100 km apart⁵⁵. Crane⁴³ noted that the spacing between areas of upwelling and igneous activity in oceanic zones of crustal accretion is related to spreading rate, that is, ~150 km along the fast East Pacific Rise, ~100 km along the intermediate Galapagos rift and Juan de Fuca Ridge, and ~50 km along the slow MAR. A regular spacing of ~40 km has been noted for active volcanoes in the eastern volcanic zone of Iceland, part of the MAR⁵⁶. The concept of regularly spaced volcanism can be extended to continental rifts: a spacing of ~40 km has been reported for Pleistocene and active volcanoes of the northern Kenya and Ethiopian rifts, and of 30–10 km for those of the Afar rift⁵⁷. The increase in spacing of hot points from continental to proto-oceanic, to slow, intermediate and fast oceanic rifts (Fig. 6) is paralleled by an inferred uplift of sub-rift isotherms and asthenosphere/lithosphere boundary, an increase in the degree of upper-mantle melting and a change in melt chemistry from alkaline to transitional to tholeiitic and to MORB. The first factor above, causing an increase in the thickness of the zone of partial melting from continental to slow to fast-spreading rifts^{43,48}, would determine a corresponding increase in the spacing of asthenospheric diapirs and volcanism. Thus, the transition from continental to oceanic rift as seen in the central Red Sea could be considered an important step in a continuum of rift processes, related ultimately to the evolution of mantle thermal waves.

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Correcting an immune-response deficiency by creating E_α gene transgenic mice

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We have introduced a cloned E_α^k gene into embryos of mice incapable of expressing their endogenous E_α genes. The transcription of E_α^k was accurate and tissue-specific in the resulting transgenic lines and, in macrophages, E_α^k transcription could be induced by γ -interferon. The injected gene conferred on the genetically deficient host strain the ability to mount an immune response to a synthetic polymer.

THE immune response to a foreign antigen involves the highly regulated expression of several specific gene sets. Mounting a successful antibody response to antigen challenge depends on mobilizing a repertoire of lymphoid cells: macrophages and dendritic cells that process and present antigen, B cells that effect the actual production of immunoglobulins and T cells that modulate the B-cell response.

A group of loci important in controlling the murine immune response have been mapped to the major histocompatibility complex (MHC) on chromosome 17 (see refs 1–5 for reviews). The 'immune-response' genes encode the Ia antigens, glycoproteins that occur at the cell surface as heterodimeric complexes, the A complex (A_α and A_β) and the E complex (E_α and E_β). Ia molecules are limited in distribution primarily to B cells and antigen-presenting cells (APC) including macrophages; certain immunological 'activators' such as γ -interferon (IFN- γ) and B-cell growth factor increase their expression. Immune-response genes are characterized by an extensive polymorphism, such that any two mouse strains have Ia chains that differ by 1–10% of their amino acids. Certain strains lack the E complex entirely.

The polymorphism of the Ia antigens seems to be the key to their function in the immune response. T cells can only 'see' foreign antigen in the context of an Ia complex and respond only when an APC 'presents' antigen in conjunction with an Ia molecule of the appropriate haplotype. Some antigens are presented in association with the E complex, others with the A, hence particular antigens are said to be E- or A-restricted. T cells activated in response to antigenic stimulation are essential for the B-cell response; this T-B interaction is also subject to 'restriction' by Ia molecules of the appropriate haplotype. The net result of joint antigen-Ia recognition by T cells is that when challenged with a given foreign antigen, antibody is produced depending on the particular Ia alleles expressed.

Recently, the genes for all four Ia chains have been cloned and multiple alleles of each sequenced². Experiments are being performed to localize the regions of each chain involved in restriction, and to define the mechanisms involved in the regulation of Ia gene expression throughout B-cell development and in response to inducers. As the immune response is a phenomenon involving interacting cells and multiple coordinately expressed gene products, we examined Ia expression and function in the whole organism by injecting Ia genes into mouse single-cell embryo pronuclei and identifying the resultant transgenic mice. Recent experiments injecting immunoglobulin, elastase and transferrin genes and some genes under the control of the metallothionein promoter have documented expression of these genes in a tissue-specific or at least a tissue-preferential fashion^{6–14}.

Here we demonstrate the use of transgenic mice in studying Ia expression and function. Mice of the $H-2^s$ or $H-2^b$ haplotypes do not express the E_α gene because of a deletion that removes its promoter region and first exon^{15,16}, consequently they cannot produce antibody in response to

E-restricted antigens such as the synthetic polymer poly(Glu⁵⁷Lys³⁸Phe⁵) (GLØ). We have injected the E_α^k gene into $H-2^{(b \times s)}$ hybrid mouse embryos. The resulting transgenic mice transcribe the E_α^k gene in a tissue-specific manner, express heightened levels of E_α in response to IFN- γ and assemble an E_α^k/E_β^s or E_α^k complex at the cell surface that can be recognized by monoclonal antibodies. Finally, as evidence of the overall efficacy of the injected E_α^k gene, the transgenic mice can produce antibody in response to GLØ.

E_α^k gene incorporation

The E_α^k gene from strain A/J mice has previously been cloned into λ Charon 4 (ref. 17). A partial restriction enzyme map of this clone is presented in Fig. 1a, which also shows the position of a 600-base pair (bp) deletion at the 5' end of the E_α gene from $H-2^b$ and $H-2^s$ mice. This deletion precludes E_α gene transcription in these strains because it includes the promoter region and the first exon¹⁶.

We injected the cloned E_α^k gene into the male pronucleus of F_2 hybrids derived from C57BL/6 $\times$ SJL hybrid crosses. (Note that SJL mice are $H-2^s$ and C57BL/6 are $H-2^b$.) This strain combination was chosen because it produces vigorous embryos⁷, is easy to manipulate and the progeny, $H-2^b$ and/or $H-2^s$, would not transcribe the endogenous E_α gene. In one experiment we injected the entire λ clone; 21 offspring developed from 88 surviving embryos, one of which (no. 23) had incorporated E_α^k . In a second experiment, we injected the purified 8.2-kilobase (kb) *Bgl*I fragment which contains all the E_α exons plus 2 kb of DNA 5' to the cap site and 1.4 kb 3' to the poly(A) addition site (see Fig. 1a). Of 273 surviving embryos, 16 were born live, including one (no. 16) that had incorporated E_α^k .

Figure 1b shows a Southern blot of tail DNA digested with *Bam*HI/*Eco*RV and hybridized with a small fragment (probe 1) entirely contained within the E_α deletion of $H-2^b$ and $H-2^s$ mice. Only the E_α^k gene should generate a signal with this probe (see Fig. 1a). Mice Ea16 and Ea23 exhibit a *Bam*HI/*Eco*RV fragment indistinguishable in size from that of A/J mice which contain two E_α^k genes per diploid genome. In contrast, Ea7 did not incorporate the injected DNA and thus provides a useful negative control.

The blot in Fig. 1c shows *Hind*III/*Eco*RV digests hybridized with probe 2, identified in Fig. 1a. Lane 1 of Fig. 1c shows the pattern expected from an E_α^k gene: a large band of ~6.6 kb containing all the E_α exons and a small band of ~1.8 kb containing sequences 5' to the coding region. The $E_\alpha^{b,s}$ patterns are seen in the next two lanes. The large band is smaller than for E_α^k because of the 600-bp deletion; the small band is larger than for E_α^k because of a polymorphic *Hind*III site (D.M. and C.B., unpublished observations). Mouse 23 exhibits all the bands characteristic of the E_α^k and $E_\alpha^{b,s}$ genes, whereas mouse 16 has the large bands diagnostic of E_α^k and $E_\alpha^{b,s}$ and the small band of only the latter. The small E_α^k band was not expected because the 5' border of the *Bgl*I fragment injected into mouse 16 is contained in the small *Hind*III/*Eco*RV fragment (see Fig. 1a).

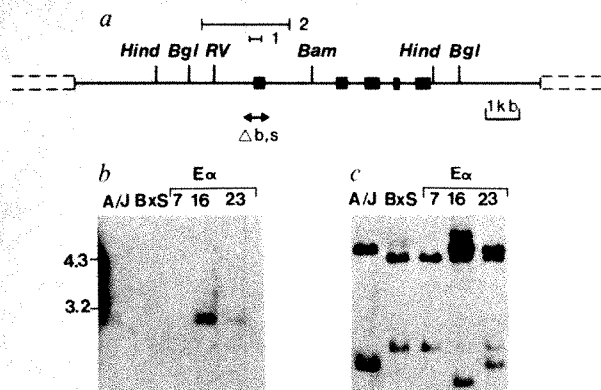


Fig. 1 Integration of the E_α^k gene into the genome of transgenic mice. *a*, Map of the E_α^k gene in the λ clone $\lambda A/J$ p34.13 (ref. 17). Thick lines, exons; thin lines, introns and flanking sequences; dashed boxes, vector arms. The deletion of the $H-2^b$ and $H-2^s$ haplotypes in E_α is shown as a double-headed arrow. Brackets indicate the two probes used. Probe 1, a 320-bp *Acc*I/*Sac*I fragment (from position -220 to +100 with respect to the major site of initiation of transcription) that does not hybridize to DNA from $H-2^b$ and $H-2^s$ haplotype mice. Probe 2, a 2-kb *Sal*I fragment spanning ~1 kb on either side of the b/s deletion. Only the relevant restriction enzyme sites are shown. *Bam*, *Bam*HI; *Bgl*, *Bgl*I; *Hind*, *Hind*III; *R*V, *Eco*RV. *b*, *c*, Southern blot analyses (described in ref. 17) of tail DNA from several mice: A/J, C57BL/6 $\times$ SJL F₁ (B $\times$ S), and mice derived from injected eggs (E α 7, 16 and 23). In *b*, DNA was digested with *Bam*HI and *Eco*RV, and the blot hybridized to probe 1. *c*, The pattern generated with *Hind*III and *Eco*RV was revealed by probe 2. The band of high relative molecular mass in E α 16 results from incomplete digestion.

It is replaced by a slightly smaller band, whose size is consistent with the notion that many of the copies of E_α^k in mouse 16 are organized as tandem repeats. This blot demonstrates first, that the injected genes are not grossly rearranged in either mouse 23 or mouse 16, as shown by the intact 6.6-kb band containing all the exons. Second, by comparing within the same slot, the band intensities of fragments derived from the injected E_α^k genes with those from the endogenous $E_\alpha^{b,s}$ genes, we can accurately quantitate E_α^k copy number. Thus, mice 23 and 16 contain, respectively, ~4 and 12 copies of the intact E_α^k gene per diploid genome in tail tissue. Similar results were obtained with liver DNA.

To ascertain whether these genes were incorporated in the germ line, we mated mice 23 and 16 with mice from strain C57BL/6 ($H-2^b$). Mouse 23 produced nine young, four of which had integrated the E_α^k gene, whereas 7 of the 16 offspring from mouse 16 had the E_α^k gene. These values suggest that, for each transgenic mouse, all copies of the injected gene had integrated into the same chromosome. Southern blots of *Bam*HI digests confirmed that the offspring from mouse 16 had inherited the same number of copies, and that this number was the same as for their parent (data not shown).

E_α^k expression

The E complex is absent from most murine cells, but is present in high concentrations on B cells and activated macrophages⁴. Thus, we chose spleen tissue for the initial analysis of transcription from the injected E_α^k gene. A Northern blot of total spleen RNA hybridized with an E_α complementary DNA probe (Fig. 2*a*) shows that transgenic mice E α 16 and E α 23 express the same 1.25-kb E_α transcripts as control mice of haplotype $H-2^{b \times a}$. It should be recalled that neither the E_α^b nor E_α^s genes are transcribed, so that all the visualized RNA derives from the E_α^k gene.

To confirm the accuracy of transcription, we mapped initiation sites on the E_α^k gene in the spleen. RNA was hybridized with a single-stranded DNA probe labelled at the 5' end. If initiation were correct, treatment of the DNA-RNA hybrids with S₁ nuclease should result in a pair of protected fragments ~100 bp

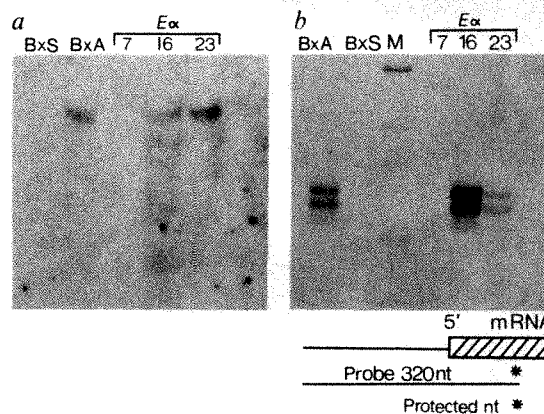


Fig. 2 Accurate expression of the integrated E_α^k gene. Total RNA was prepared from spleens of different control or transgenic mice and analysed by Northern blotting (*a*) or S₁ nuclease mapping (*b*). The nomenclature for the mouse strains is described in Fig. 1 legend, except for B $\times$ A which is a C57BL/6 $\times$ A/J F₁ hybrid. The filter in *a* was probed with an E_α cDNA clone¹⁷. In *b*, the probe was a 320-nucleotide (nt) single-stranded DNA fragment (*Acc*I/*Sac*I; see Fig. 1 legend). Hybridization with E_α mRNA protects a set of fragments ~100 nucleotides long, which was revealed by electrophoresis on a urea-acrylamide gel followed by autoradiography. M, relative molecular mass markers with sizes of 147, 122, 110, 90 and 76 nucleotides. Techniques and conditions have been described elsewhere¹⁷.

long, as is seen in Fig. 2*b* for a B $\times$ A mouse. The complexity of the pattern results from multiple initiation sites (see discussion in ref. 17). In spleen RNA from mice 16 and 23, initiation on the E_α^k gene was accurate. Mouse 23 seems to produce fewer transcripts than the B $\times$ A hybrid, whereas mouse 16 makes more. These differences are more apparent than in the Northern blot, probably because the S₁ assay is less sensitive to partial RNA degradation.

The specificity of transcription was evaluated by S₁ analysis of RNA from several other tissues (Fig. 3). In the B $\times$ A control, high levels of E_α messenger RNA were observed in spleen and lung tissue, lower levels were detected in the kidney and almost no E_α mRNA could be measured in the liver, heart and brain. The results with lung and kidney are somewhat surprising in view of the contention that Ia genes are expressed mainly on B cells and macrophages. However, Northern blots of lung and kidney RNA hybridized with an immunoglobulin κ -chain probe indicated that these tissues are contaminated by lymph nodes, macrophages and/or significant blood infiltration (data not shown; ref. 14). Transgenic mice E α 16 and E α 23 exhibit the same pattern of tissue distribution: high levels in spleen and lung and low or undetectable levels in kidney, liver, heart and brain (Fig. 3). This S₁ assay may not detect aberrant transcripts initiating in regions of the E_α gene not covered by the rather short probe. To exclude this possibility, we also ran Northern blots on RNA from the different tissues and did not detect anomalous bands, confirming the S₁ mapping results (data not shown).

Another method of assessing tissue-specific expression of the injected gene is to compare the amount of A_α and E_α^k transcripts in various tissues. A_α has a high level of sequence homology with, an identical tissue distribution to, and a functional role analogous to the E_α chain. In S₁ experiments similar to that illustrated in Fig. 3, RNA was hybridized simultaneously with E_α and A_α probes (see Fig. 3 legend). Clearly, the bands attributed to E_α mRNA (100 bp) have the same pattern of distribution as those resulting from A_α transcripts (128 and 260 bp). We concluded that the injected E_α^k gene is transcribed coincidentally with the endogenous A_α gene in the six tissues studied. In other experiments, we have confirmed this tissue-specificity of expression in one offspring from each of mice 16 and 23.

Evidence for the tissue-specificity of transcription is

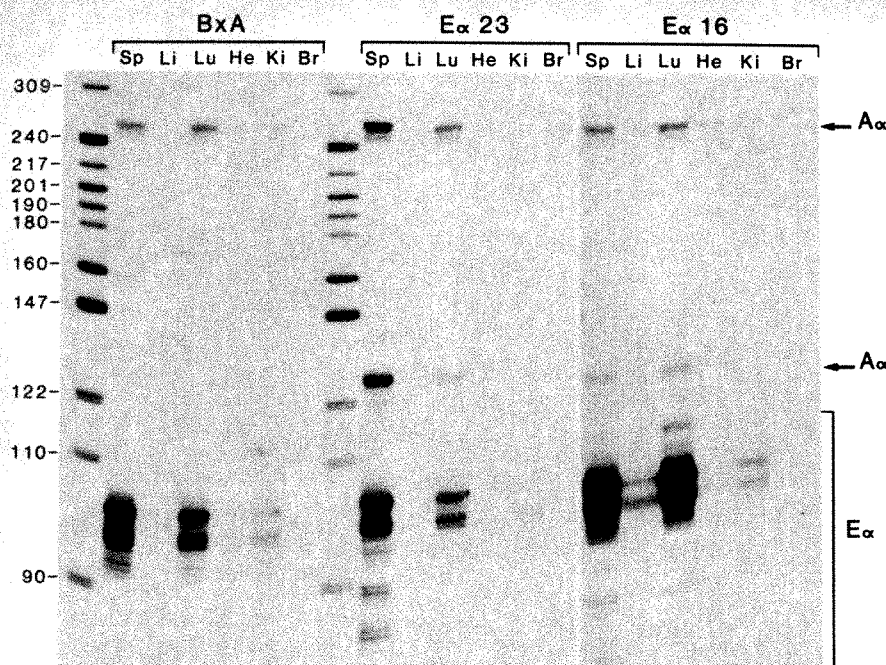


Fig. 3 Tissue-specific expression of E_{α}^k in transgenic mice. E_{α} and A_{α} mRNA was analysed in various tissues from three mice by S_1 nuclease mapping of total RNA. For E_{α} , the probe was the same as in Fig. 2. For A_{α} , the probe was an 800-bp *Hind*III fragment from an A_{α}^b cDNA clone, which was labelled at the *Hind*III site at position 262 and extended through the first domain, the leader and the plasmid sequences. A_{α}^b mRNA protects a 262-nucleotide fragment. The second A_{α} band on the gel results from an internal S_1 nuclease cut when the probe is hybridized to A_{α}^b mRNA; allelically polymorphic residues of the s haplotype²¹ locally destabilize the hybrid enough for partial S_1 nuclease cleavage under our conditions. The tissues investigated were spleen (Sp), liver (Li), lung (Lu), heart (He), kidney (Ki) and brain (Br), from a C57BL/6 $\times$ A/J F_1 hybrid (B $\times$ A), and from E α 16 and E α 23.

thus twofold: first, E_{α}^k expression in transgenic mice closely parallels that in the B $\times$ A control; second, transcription of the E_{α}^k and the endogenous A_{α} genes follows a very similar pattern. However, because some of the tissues exhibit a high background resulting from blood infiltration, macrophages or lymph nodes, we cannot eliminate the possibility that the transgenic mice exhibit low levels of aberrant transcription in these tissues. This might be the case in mouse 16 liver, where a low level of E_{α} mRNA (a few per cent of that seen in the spleen) is observed.

As a measure of cell-type specificity of expression, we compared the distribution of the A and E complexes on spleen cells by labelling them with fluorescent anti-E and anti-A monoclonal antibodies and visualizing them by two-colour cytofluorometric analysis. Expression of the A complex on the cell surface depends on transcription of the endogenous A_{β} and A_{α} genes; expression of E requires transcription of the endogenous E_{β} and the injected E_{α} genes. Thus, we could not ask whether E_{α}^k was transcribed in cells in which the other Ia chains did not occur because we would not detect it on the cell surface (resulting from the absence of E_{β}). However, we could determine whether cells expressing the A complex (and thus also transcribing the endogenous E_{β}) all exhibit an E complex on the cell surface. Figure 4 shows that the spleen cells of one of the progeny of mouse 16 consist almost entirely of two populations: $A^{-}E^{-}$ and $A^{+}E^{+}$. As expected, there is no $E^{+}A^{-}$ component. The fact that there is no significant $A^{+}E^{-}$ population indicates that essentially all cells that synthesize the endogenous A_{α} , A_{β} and E_{β} chains also transcribe E_{α}^k . A few cells trail into the $A^{+}E^{\text{dull}}$ area, but this minor component can also be seen in spleens from B $\times$ A controls.

E_{α}^k gene induction

Expression of all four Ia antigens is stimulated greatly in macrophages by treatment with supernatants from activated T cells or with purified IFN- γ . This induction was first described for cell-surface Ia molecules (see ref. 18 for review), but more recently has been documented for stable mRNA transcripts^{17,19}. To determine whether the injected E_{α}^k gene was similarly sensitive to IFN- γ induction, we prepared peritoneal macrophages from E α 16 and E α 23 third-generation mice, cultured them in the presence or absence of recombinant IFN- γ for 60 h and quantitated A_{α} and E_{α} mRNA levels by S_1 mapping.

Figure 5 clearly shows an induction of A_{α} and E_{α}^k mRNA in the IFN- γ -treated macrophages from both transgenic mice. The levels of Ia induction are similar to those that we have observed for E_{α}^k in repeated experiments on other mice.

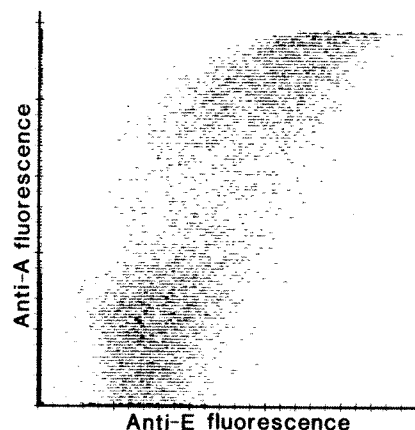


Fig. 4 I-A-positive spleen cells are also I-E-positive in the E α 16 transgenic line. Twenty hours after concanavalin A activation, spleen cells were stained with fluorochrome-labelled monoclonal antibodies specific for the A or the E complex. For each cell, red and green fluorescence was detected by flow cytofluorometry. On this two-parameter representation, single cells appear as thin dots; thicker dots indicate several cells in the same channel.

Methods. Antibodies were purified from ascites fluid by affinity chromatography on protein A-Sepharose (Pharmacia). The anti-I-A antibody BP107 (ref. 22) was directly conjugated to fluorescein isothiocyanate (FITC) as described elsewhere²³. Antibody 14.4.4, specific for the E complex, was conjugated to phycoerythrin (PE; Molecular Probes, Ore) according to Oi *et al.*²⁴. After purification on Lympholyte M (Cedarlane), lymphocytes were stained in one step in FWS (phosphate-buffered saline supplemented with 3% calf serum, 30 mM HEPES and 0.1% sodium azide). Incubation was for 30 min on ice with saturating amounts of antibody. After two washes in FWS, the cells were examined on an ATC 3000 flow cytometer (ODAM, Wissembourg) equipped with an Innova-90 argon laser set on the 488-nm line for excitation of both fluorochromes. Signals from lymphocytes were selected by electronic gating according to size (impedance measurement) and laser scatter. Emission from the fluorochromes was separated by a 555-nm SWP017 dichroic mirror (Melles Griot, Irvine) and further elimination of signal cross-contamination was accomplished with a 550-nm SWP015 short-pass filter in front of the FITC detector and a 575-nm 5151 long-pass filter (Oriol, Stratford) in front of the PE detector. A small degree of electronic compensation was also applied before logarithmic amplification. Average channel numbers on the red and green scales were: $A^{-}E^{-}$, 73 and 52; $A^{+}E^{+}$, 160 and 210; unstained cells (or cells stained with irrelevant antibodies) were centred around channels 71 and 45 in the red and green scales, respectively.

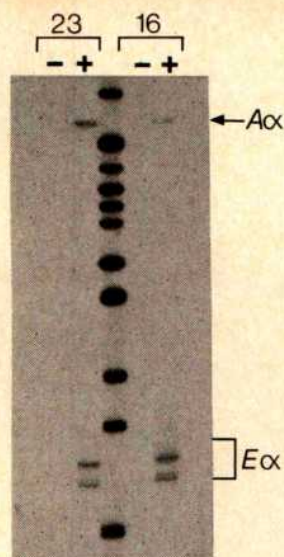


Fig. 5 Injected E_{α}^k gene response to IFN- γ . Peritoneal macrophages were elicited with thioglycolate (3 days)²⁵ and subsequently cultured for 60 h in the presence (+) or absence (-) of 100 U ml⁻¹ recombinant IFN- γ . RNA was prepared and analysed by S₁ nuclease mapping as described in Fig. 3 legend. The mice were from the F₂ of a backcross to C57BL/6.

Functional E_{α}^k gene product

The ability of an animal to make antibody in response to the synthetic antigen GL $\emptyset$ is dependent on expression of the E complex. The mice that served as hosts for the injected E_{α}^k gene do not respond to GL $\emptyset$ because they are the progeny of (H-2^b $\times$ H-2^s) hybrid crosses and thus do not express any E_{α} . However, if the injected E_{α}^k gene makes a functional product, the animal should be able to mount a response to GL $\emptyset$. To test this possibility, we injected some of the offspring of mice 16 and 23 with GL $\emptyset$ according to the protocol described in Fig. 6 and evaluated their secondary response by quantitating serum levels of anti-GL $\emptyset$ antibody. Note that these offspring derive from a C57BL/6 $\times$ transgenic mating, so only about half carry the injected E_{α}^k gene. Ovalbumin was injected at the same time to serve as a positive control; as it is an A-restricted antigen, b- and s-haplotype mice can respond.

Figure 6 shows a perfect correlation between the presence of the E_{α}^k gene and the ability to make antibody in response to GL $\emptyset$. The titres and mRNA levels for the offspring of mouse 16 are generally higher than those of mouse 23 (Figs 2b, 3). There is some variability in the response for different mice from the same transgenic parent, but we have also seen variable antibody titres for the B $\times$ A controls, and thus we do not lend much significance to the range of responses. The lower values cannot be attributed to the H-2 haplotypes (see Fig. 6, beneath the histogram), to a difference in the number of E_{α}^k genes that have been inherited (data not shown) or to general immunodeficiency (because of a healthy ovalbumin response).

Conclusion

We have injected a cloned E_{α}^k gene into embryos of mice incapable of expressing their endogenous E_{α} genes. The cloned DNA was incorporated into the germ line and inherited in a normal mendelian fashion. Expression of the E_{α}^k gene is tissue-specific, similar to transcription of the endogenous homologue, A_{α} . In peritoneal macrophages, E_{α} mRNA synthesis is responsive to induction by IFN- γ . The injected gene is transcribed accurately and specifically in several cell types, and the animals can mount a normal immune response.

Our results show that tissue-specific expression and inducibility by IFN- γ of the E_{α} gene is conferred by sequences within or relatively close to the structural gene: mouse 16 was injected with an 8.2-kb fragment containing only 2 kb upstream from the cap site and 1.4 kb downstream to the poly(A) addition site.

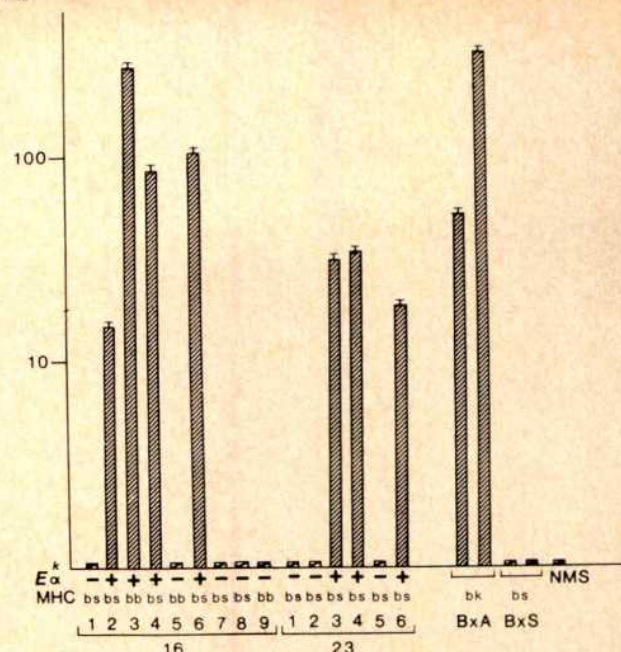


Fig. 6 E_{α}^k -positive transgenic mice mount an effective immune response to the synthetic antigen GL $\emptyset$. Fifteen mice, derived from crossing E α 16 or E α 23 with C57BL/6, were challenged with the terpolymer (Glu-Lys-Phe), and specific antibody titres were measured in a sandwich enzyme-linked immunosorbent assay. Bars represent the values (in arbitrary units) obtained for the different offspring and for control B6 $\times$ A/J or B6 $\times$ SJL F₁ hybrids. MHC haplotypes are shown below the E_{α}^k -status, and were determined for the transgenics by Southern blotting of genomic DNA cut by PstI and probed with an A_{α} cDNA fragment. PstI generates polymorphic A_{α} bands which enable the H-2^b and H-2^s haplotypes to be distinguished. NMS, normal mouse serum.

Methods. Mice were taken at 2 months old and were injected intraperitoneally at days 1 (in complete Freund's adjuvant) and 21 (in saline), each time with 25 μ g GL $\emptyset$ and 25 μ g chicken ovalbumin (OVA). Sera were collected at day 28 and tested for specific antibodies by incubating dilutions in microtitre plates previously coated with GL $\emptyset$ or OVA. After washing off unbound antibodies, we added to the wells an alkaline phosphate-conjugated goat anti-mouse immunoglobulin antibody (Cappel); this was followed after 2 h by extensive washing. Bound enzyme was measured by the rate of conversion of p-nitrophenyl phosphate, with several absorbance readings taken on a Titertech Multiscan. Titres were calculated from the linear portions of the curves obtained, and are expressed in arbitrary units by comparison with a laboratory standard serum.

Therefore, in future experiments it will not be necessary to inject large cosmid clones that may contain adjacent genes.

By the injection of a single gene we have endowed a genetically deficient host strain with a new immune response. A successful antibody response implies the involvement of the E complex in various cell/cell interactions, for example, APC-T cell, T-B cell. In addition, T cells must be 'educated' to the E complex in the thymus. That the E_{α}^k gene product seems to function correctly in all the necessary immunological contexts allows the study of Ia function in the organism. The importance that mutant and recombinant mice have already had in studies elucidating the intricacies of the immune response cannot be underestimated. The generation of such strains is, however, limited to fortuitous mutations or crossovers, and unknown genetic segments can be carried along during the construction of lines (see, for example, ref. 20). It should now be much easier to create mouse strains which differ by only a single gene.

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LETTERS TO NATURE

On the nature of the companion to Van Biesbroeck 8

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We present here the results of the first numerical evolutionary calculations for very low-mass stars (masses in the range of $0.01-0.1 M_{\odot}$) with ages up to the age of the Galaxy. Our calculations shed new light on the nature of the recently discovered object VB 8B (ref. 1), the companion to Van Biesbroeck's star no. 8 (VB 8; ref. 2), and support the identification¹ of this object as a low-mass star in which hydrogen burning was never able to establish thermal equilibrium. For plausible ages of this binary stellar system ($\geq 10^9$ yr), we estimate the mass, M , of VB 8B to be in the range $0.04-0.08 M_{\odot}$ (see also ref. 1 and refs therein). The object is supported primarily by degenerate electron pressure, and we find that its present values of central density and temperature are $\rho_c \approx 500-2,000 \text{ g cm}^{-3}$ and $T_c \approx 1.0-1.5 \times 10^6 \text{ K}$, respectively. The star VB 8 itself³⁻⁵ may also be a low-mass star which marginally failed to establish main-sequence hydrogen burning. Thus, the mass ratio for this binary system is probably not greatly different from unity, and we predict an orbital period of ≥ 40 yr. Our evolutionary tracks are also applicable to the interpretation of observations of other very low-luminosity dwarfs (see refs 3, 5-7). The study of such objects may be directly relevant to the development of an understanding of the 'dark matter' that has been inferred, from dynamical considerations, to be present in our Galaxy and many extragalactic systems (see, for example, refs 8, 9).

Several stars have been found to have either a mass close to or below that of the end of the hydrogen-burning main sequence (for example, Luyten 726-8A and B; Ross 614B; refs 6, 7, 10-12), or an effective temperature below that expected for even the lowest mass main-sequence star (for example, LP 271-25 = LHS 2924; ref. 5). Some of these stars are in binary systems whereas others have no detected companion. Such stars may be of sufficiently low mass ($\leq 0.08 M_{\odot}$ for a hydrogen-rich star^{13,14}) that they never achieve thermal equilibrium through hydrogen burning, and are candidates for 'brown dwarfs'. This is something of a misnomer, in that some stars with masses below the minimum main-sequence mass can attain effective temperatures quite comparable with those of ordinary late M dwarfs for a substantial interval of time ($\geq 3 \times 10^8$ yr) during their contraction phase. The existence of very low-mass ($\leq 0.01 M_{\odot}$), unseen companions to nearby stars has also been inferred by astrometric

techniques^{15,16}, but the reliability of these detections remains uncertain (see ref. 17).

In addition, the presence of low-mass ($M \leq 0.1 M_{\odot}$) stars has been inferred in several interacting binary systems, including two binary X-ray sources and several cataclysmic variables with orbital periods near the minimum in their orbital period distribution (see refs 14, 18, 19 and refs therein). In all cases, however, these binary systems are studied in X-radiation and/or visible light produced by the accretion of matter from the hypothesized low-mass secondary to a collapsed star (degenerate dwarf or neutron star). The low-mass star itself is not directly detected. Furthermore, in these systems, the secondary stars are thought to have attained their present low-mass state by transferring a large amount of their original mass to the collapsed star (or by loss of mass from the binary altogether). Thus, most of these low-mass interacting stars are probably not primordial: that is, they have evolved from more massive objects.

To get a better understanding of the object VB 8B, which is reported to have a radius, R , of $\sim 0.09 R_{\odot}$ and an effective temperature, T_e , of $\sim 1,360 \text{ K}$ (ref. 1), we have carried out evolutionary calculations for stars of mass $\leq 0.1 M_{\odot}$. We utilized a simplified stellar evolution code developed by Rappaport *et al.*¹⁹ and modified by Rappaport and Joss¹⁴ to carry out the present calculations. The basic simplifying features of the code are in (1) the treatment of the stellar structure, which is taken to be completely convective and is represented by an $n = 3/2$ polytrope, and (2) the matching of entropy in the stellar interior directly to that at the photosphere. The former approximation has been shown to be adequate^{6,13,18-20} for a low-mass star during its cooling from very early evolutionary stages all the way to a totally degenerate configuration. The assumption of matched entropies has been utilized successfully in the past¹⁴, and we have carried out further checks in the present work (see below). The code we utilize, however, cannot handle cold objects of mass $\leq 0.005 M_{\odot}$. For the present study, we have slightly modified the code to include the burning^{7,21} of a small quantity (5×10^{-5} by mass; see ref. 22) of primordial deuterium, and an approximate expression which incorporates the Coulomb corrections to the equation of state^{23,24}. This latter expression was adjusted so that the stellar radii we obtain for totally degenerate configurations almost exactly match the results of more detailed structure calculations by Zapolsky and Salpeter²⁵. Unlike some previous calculations of cooling curves for very low-mass stars (see 6, 7, 26 and refs therein), our evolutionary runs are continued to very low effective temperatures and the behaviour of the parameters is presented explicitly as functions of time. Stevenson²⁷ has carried out semi-analytical calculations of the cooling of degenerate dwarfs to very low temperatures, but, unlike the present study, his calculations did not follow the evolution of the stellar radius.

We considered stellar masses in the range of $0.01\text{--}0.10 M_{\odot}$, in increments of $0.01 M_{\odot}$. We note, that $0.01 M_{\odot}$ is an especially plausible operational definition of the dividing line between 'stars' and 'planets', because this is about the minimum mass for which the pressure support in the final equilibrium configuration is contributed principally by either thermal gas pressure or degenerate electron pressure, as opposed to atomic and molecular forces. Moreover, $0.01 M_{\odot}$ is about the minimum mass for which quasi-equilibrium thermonuclear fusion (in the form of deuterium burning) can ever be established (see below).

We tested two chemical compositions, one appropriate to Population I stars $[(X, Y, Z) = (0.70, 0.28, 0.02)]$ and the other appropriate to Population II $[(X, Y, Z) = (0.719, 0.28, 0.001)]$. We used three sets of radiative opacities; one^{19,28} simulated the effects of grain formation in the stellar atmosphere at low temperatures ($T_e \leq 2,200$ K; hereafter, opacity AG), while the other two, which ignored such effects, were adapted from the opacity tables derived by Alexander^{19,28} and the fitting formulae, due to Webbink (ref. 14 and personal communication), based on the Cox-Stewart²⁹ and Cox-Tabor³⁰ opacities (hereafter, opacities A and W, respectively). The effect of possible grain formation on the radiative opacities at low temperatures was approximated by setting the numerical value of the Alexander^{19,28} opacity coefficient equal to a constant for $T_e \leq 2,200$ K (see also ref. 18).

All initial stellar models had $R \geq 1 R_{\odot}$, which is at least 10 times greater than the radius of any plausible model of VB 8B, and were assumed to have negligible rotational angular momentum. If, however, the angular momentum were not significantly dissipated by the time the contracting protostar had reached a radius of $\sim 1 R_{\odot}$, then the early portions of the evolutionary cooling tracks could be quite different from those we present. On the other hand, it is quite plausible that some form of braking torque, possibly associated with a magnetic stellar wind (see ref. 31), will effectively despin the star, so that for stellar ages $\geq 10^7$ yr our evolutionary calculations should be valid. (For a recent review of some of the processes involved in star formation, see ref. 32 and refs therein.)

Figure 1 shows cooling curves for stellar models of several different masses; Fig. 1a and b show the evolution of the effective temperature and stellar radius, respectively. All models in Fig. 1 were generated with a Population I chemical composition and the AG radiative opacities. Note that the contraction of each stellar model is temporarily halted for times between $\sim 10^6$ and $\sim 10^7$ yr due to deuterium burning (see also ref. 7). After deuterium exhaustion, contraction resumes towards the final equilibrium state. For $M \geq 0.086 M_{\odot}$, the star achieves a state of thermal equilibrium (that is, arrives on the hydrogen-burning main sequence) within $\sim 10^9$ yr and has an effective temperature far greater than that measured for VB 8B [$1,360 \pm 200$ K (ref. 1)]. For lower mass stars, thermal equilibrium is never reached (except temporarily on the deuterium-burning main sequence) and the stars cool indefinitely. However, for a star whose mass is only slightly lower than the minimum mass for the hydrogen-burning main sequence, cooling times required to achieve $T_e \leq 2,000$ K may exceed the age of the Universe. We note that for $T_e \leq 2,000$ K the atmospheric radiative opacities become highly uncertain; however, we have found that the cooling timescales are rather insensitive to changes in the assumed opacity law.

We have repeated the evolutionary calculations presented in Fig. 1 for the other radiative opacities discussed above and for stars with a Population II chemical composition. The results are in qualitative agreement with those shown in Fig. 1. The implementation of opacity A has the effect of initially decreasing the cooling rate and then increasing the rate at later times. We found that the greatest overall change in the cooling timescale, relative to that obtained with the AG opacities, was about a factor of 3 and occurred for the $M = 0.01 M_{\odot}$ model. More typically, for other masses, the cooling times changed by a factor of < 2 . When opacity W is used, the change in the behaviour of the cooling rate is similar to that found for opacity A, and the effective temperatures are systematically raised by $\sim 10\text{--}15\%$ during the initial contraction phase. As a further test of the

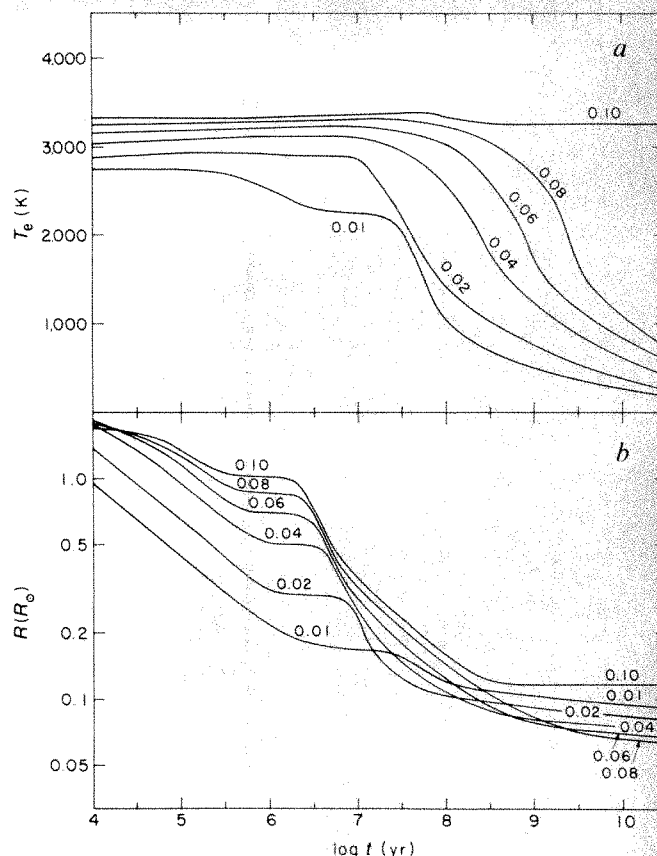


Fig. 1 Evolutionary tracks for very low-mass stars with masses in the range of $0.01\text{--}0.10 M_{\odot}$. The effective temperature, T_e , and stellar radius, R , are shown as functions of elapsed time, t , in *a* and *b*, respectively. Each track is labelled with the corresponding stellar mass, in units of $M_{\odot}$. The plateau regions evident in the plots of radius versus time (near $t = 10^6\text{--}10^7$ yr) are due to the burning of a small quantity (5×10^{-5} by mass) of primordial deuterium. Stars with masses in excess of $\sim 0.086 M_{\odot}$ ultimately achieve thermal equilibrium through hydrogen burning (that is, they attain hydrogen-burning main-sequence status). Stars of lower mass cool indefinitely towards a completely degenerate configuration. For $T_e \leq 1,000$ K the displayed cooling curves become increasingly uncertain. Our values of T_e for $t \geq 3 \times 10^9$ yr are in good agreement with those calculated by Stevenson²⁷.

sensitivity of our results to the values of the radiative opacities at low temperatures, we have calculated several series of cooling curves where we adopted various (constant) values for the opacity coefficient at $T_e < 2,200$ K. We find that for changes in this constant opacity of up to two orders of magnitude, the cooling times change by only a factor of ≤ 2 . We caution, however, that extremely large changes in the opacity law might alter the stellar structure and surface-boundary conditions sufficiently to invalidate the simplifying assumptions that we have utilized in our code. Finally, in a similar series of tests, we have verified the insensitivity of our results to the assumption that the entropy in the stellar interior is equal to that at the photosphere.

We note that additional complications in the physics of the stellar interior, such as crystallization into a Coulomb lattice (see ref. 33) within some layers of the star, may occur at the lowest effective temperatures we consider. We have checked, in a self-consistent manner, that such effects should not affect our basic results obtained with the AG opacities for ages of $\leq 10^{10}$ yr. The effects of crystallization become important earlier during the evolution when the A or W opacities are used than when the AG opacities are employed.

If we assume that VB 8B is not exceptionally young (that its age is $\geq 10^9$ yr; ref. 1), then we can conclude from Fig. 1 that its mass must exceed $\sim 0.04 M_{\odot}$ in order for it not to have cooled below its current effective temperature. (However, observational

selection might favour the discovery of such objects when they are young and thus relatively bright; if this is the case, somewhat smaller values for the age of VB 8B might be allowed.) The effective temperature of a star with a mass $\geq 0.086 M_{\odot}$ is always much higher than that observed for VB 8B. For stars with masses in the range of ~ 0.04 to $\sim 0.08 M_{\odot}$, the radii and effective temperatures will be consistent with those observed for VB 8B during a substantial interval of time. Table 1 lists some of the physical properties of our stellar models in this mass range that have radii and effective temperatures similar to those of VB 8B.

It seems quite reasonable that VB 8 itself is also an extremely low-mass star which has a mass very close to that of a star at the end of the hydrogen-burning main sequence. According to Probst and Liebert⁵, its effective temperature is $\sim 2,400$ K. [We note, however, that temperature determinations in this range are highly uncertain, and the effective temperature of VB 8 could be somewhat higher (see ref. 1).] Table 1 also lists the properties of our stellar models with temperatures close to that observed for VB 8. A star with a mass of $\sim 0.08 M_{\odot}$ and an age of $\sim 2 \times 10^9$ yr would have properties consistent with those observed for VB 8 (refs 2–5). The absolute bolometric magnitude of such a star is 14.2. This value, together with the observed visual magnitude of ~ 16.9 (refs 2, 3) and the measured distance of 6.5 pc (ref. 2), implies a bolometric correction of -3.7 , a highly plausible value for a star with T_e in the range of $\sim 2,400$ – $2,700$ K (see refs 6, 11, 34). If we adopt masses of $\sim 0.06 M_{\odot}$ and $\sim 0.08 M_{\odot}$ for VB 8B and VB 8, respectively, and take the orbital separation to be $\geq 9 \times 10^{13}$ cm (ref. 1), we find that the orbital period of the system is ≥ 40 yr. Thus, the properties of this system are entirely consistent with those of a non-interacting binary consisting of a pair of stars whose current masses have not varied from their initial values.

Very low-mass stars have been suggested³⁵ to be a major component of the 'dark matter' that has been inferred, from dynamical considerations^{36,37}, to comprise about half the mass density in the vicinity of the Sun. Estimates of the space density of such stars, based on extrapolation from the observed numbers of low-mass stars that are on the hydrogen-burning main sequence (see, for example, ref. 38), can plausibly account for this 'missing mass'. Detailed studies of the detectability of such objects with ground-based instruments or future space observatories (such as the Hubble Space Telescope and the Space Infrared Telescope Facility) have been carried out by, for example, Staller and de Jong³⁸. The present work has yielded

more reliable cooling curves for very low-mass stars, so that this type of study should be repeated; we are carrying out such investigations and shall report our results elsewhere.

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A radio jet associated with the supernova remnant G332.4+0.1 (Kes 32)

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We report here new observations at 843 MHz of the galactic supernova remnant (SNR) G332.4+0.1 (Kes 32). They reveal what appears to be a fairly typical SNR, with a 'jet-like feature' emerging from it and expanding into an extensive 'plume' of low brightness emission. It is as though matter confined within the main body of the remnant has broken out through a nozzle and expanded into the plume. However, measurements at 8.4 GHz show the narrow part of the jet to have a relatively flat spectrum and no detectable polarization. Hence, if actually associated with the remnant, the feature is probably either a jet of particles from a central stellar remnant or a pre-existing gas structure ionized by the progenitor star.

The observations of G332.4+0.1 (Kes 32)¹ form part of a high-resolution, high-sensitivity study of SNR morphology using the University of Sydney Molonglo Observatory synthesis telescope (MOST)². At the declination of G332.4 (-60°) the telescope has a synthesized half-power beamwidth of 44 arc s (east-west) by 57 arc s (north-south). The effects of a -8%

Table 1 Models for VB 8 and VB 8B

M ($M_{\odot}$)	R ($R_{\odot}$)	T_e (K)	L ($10^{-4} L_{\odot}$)	t (10^9 yr)	ρ_c (g cm^{-3})	T_c (10^6 K)	D
VB 8B							
0.01	0.130	1,360	0.52	0.07	40	0.49	1.9
0.02	0.103	1,360	0.33	0.12	150	0.71	2.9
0.03	0.091	1,360	0.26	0.31	330	0.87	3.7
0.04	0.083	1,360	0.21	0.65	570	1.02	4.5
0.05	0.078	1,360	0.19	1.16	880	1.13	5.2
0.06	0.074	1,360	0.17	1.87	1,240	1.25	5.9
0.07	0.070	1,360	0.15	2.94	1,660	1.36	6.5
0.08	0.068	1,360	0.14	5.16	2,140	1.46	7.1
0.085	0.067	1,360	0.13	22.90	2,380	1.53	7.2
VB 8							
0.02	0.131	2,400	5.14	0.03	70	0.85	1.8
0.03	0.110	2,400	3.62	0.07	180	1.18	2.2
0.04	0.098	2,400	2.87	0.14	350	1.46	2.5
0.05	0.090	2,400	2.45	0.27	560	1.73	2.8
0.06	0.085	2,400	2.16	0.47	820	1.97	3.1
0.07	0.080	2,400	1.92	0.78	1,140	2.20	3.4
0.08	0.076	2,400	1.73	1.68	1,490	2.42	3.7
0.085	0.074	2,400	1.64	18.70	1,730	2.46	3.9

The column headings are mass (M), radius (R), effective temperature (T_e), bolometric luminosity (L), age (t), central density (ρ_c), central temperature (T_c), and ratio (D) of total pressure to thermal pressure in the stellar interior.

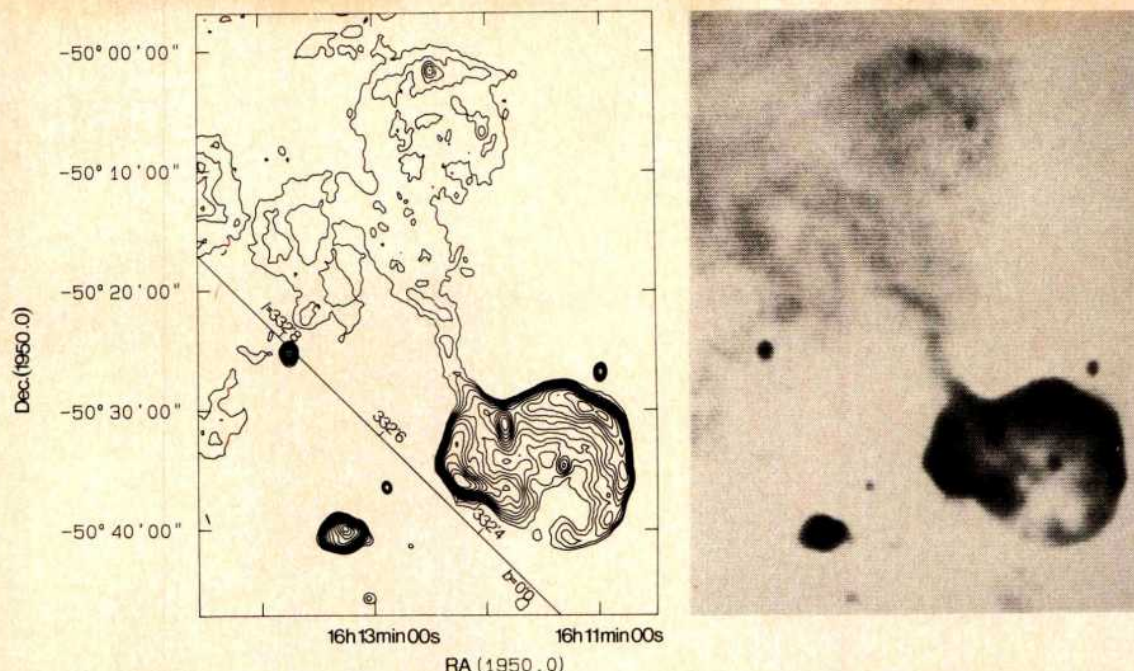


Fig. 1 Contour and grey-scale representations of the 843-MHz radio emission from the SNR G332.4+0.1 (Kes 32) and the jet-like feature emerging from the north-east side of the remnant. Contour levels are: 38, 51, 65, 81, 100, 120, 150, 190, 230, 280, 340, 410 and 500 mJy per beam area.

sidelobe, intrinsic to the mapping process, are removed by cleaning³.

Figure 1 shows contour and grey-scale maps of the 843 MHz emission from the region of G332.4. The remnant is obviously a shell source—albeit irregular in outline and in emission intensity. The two brightest parts of the shell occur on the sides of lowest and highest galactic latitudes. The west rim of the SNR has an almost circular outline, with a weak point source near its centre. The east rim is less regular and the shell appears to have a small radius.

From a point of relatively weak rim emission on the north-east side of the SNR we see a jet-like feature emerging at an angle of $\sim 20^\circ$ to the galactic plane. Near the remnant the 'jet' is narrow, kinked and most intense, whereas further from the remnant the feature broadens to form a 'plume'. At the lowest levels of emission it is difficult to discern where the plume begins to blend with unrelated neighbouring emission.

Complementary observations at 8.4 GHz with the Parkes radio telescope (3 arc min beam) (Fig. 2) show the SNR to have a similar structure to that observed at 843 MHz, with a non-thermal spectral index, which varies over the source in the range -0.45 to -0.55 with an estimated error of 0.05. These measurements, also show the emission from the north-west and south-east rims to be linearly polarized at 8% and 3.5% respectively and at position angles near 135° and 0° . The 8.4-GHz observations extend over only the narrow part of the jet-like feature. A comparison of the two maps shows this part to have a spectral index of -0.05 ± 0.1 ; no polarization could be detected to a limit of $\sim 4\%$.

The distance to G332.4 is uncertain. A lower limit is suggested by the fact that it has no detectable optical or X-ray emission⁴, whereas RCW 103, a SNR some 30 arc min away is detected in these bands⁴; the distance to RCW 103 has been set at 3 kpc (ref. 5). An upper limit of ~ 7 kpc comes from a weak OH absorption⁶, and from the 'surface-brightness-diameter' relation⁷. We have adopted 5 kpc for the calculations that follow. At this distance the diameter of the SNR is ~ 25 pc, and its age, based on the standard Sedov expansion relations, is $\sim 5,000$ yr. On the assumption that the jet and SNR are associated, the narrow part of the jet is ~ 16 pc in length and ~ 3 pc in width. The projected distance from the SNR to the top of the plume is ~ 35 pc.

The association of the jet-like feature with the SNR rests most

strongly on the fact that its emission is strongest and narrowest at the point where it meets the edge of the remnant. The appearance of the jet strongly suggests a nozzle, with material from the interior of the remnant streaming out. However, we have misgivings about such an interpretation, because of the flat spectrum and lack of polarization in the jet, and because of the theoretical difficulty in constructing the rigid skin necessary for the nozzle formation. We have therefore explored two alternative models.

In the first model, we postulate that the jet and plume may comprise synchrotron radiating particles similar in origin and energy spectrum to those which make up the centrally-bright (Crab-like) components of SNRs such as CTB 80 (ref. 8) and G326.3–1.8 (ref. 9). The brightness of the jet implies minimum (equipartition) energy densities of the order of 10^{-10} erg cm⁻³ in the narrow section and a factor of 10 lower in the extended plume. The energetics of the jet do not appear to be a problem, but the collimation may be, given the small opening angle. One possibility could be a supercritical (thick) accretion disk surrounding the central source (see ref. 10); in an alternative scheme, proposed by Benford¹¹, the pulsar provides the collimation. We note, however, that there is no suggestion of a counter jet, whereas both these schemes are inherently symmetric. Further, because of the kinked nature of the jet, further external confinement seems likely. This may be provided by static pressure in the surrounding medium or by self-generated azimuthal magnetic fields, as suggested for the jet in the Crab nebula¹¹. Broadening of the jet and formation of the plume would happen at the point where confinement breaks down owing to the interaction of the flow with the surrounding medium.

The relatively flat spectrum in the narrow part of the jet would suggest that the relativistic particles are recent in origin and, like the centrally bright components of SNRs such as G326.3–1.8, have suffered little differential loss of higher-energy particles. This short time scale implies that velocities of the order of 10^4 km s⁻¹ in the jet itself are required for this material to be transported to the distances observed.

As a second hypothesis, we suggest that the feature might be thermal material. Here we envisage a neutral gas cloud, similar to those seen as extended, narrow (probably columnar) dark clouds, that has been ionized by the pre-supernova. If stellar UV emission were the only source of ionizing radiation, one

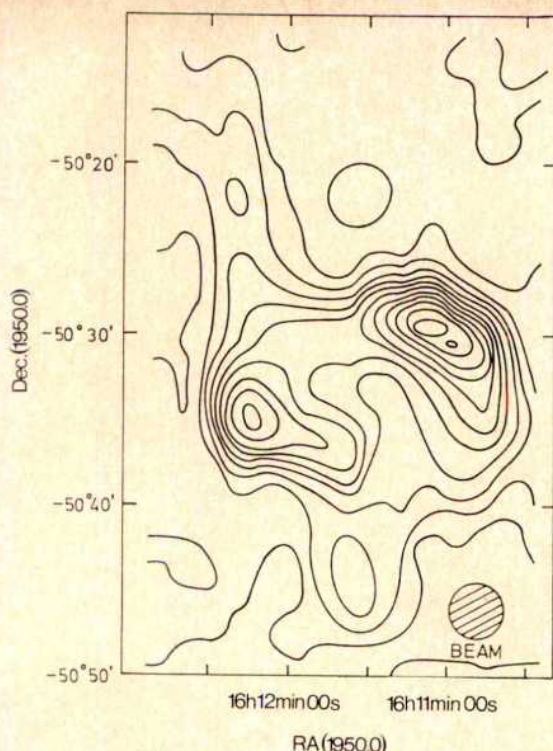


Fig. 2 Contours of the 8.4-GHz radio emission from G332.4+0.1. The contour levels are in increments of 105 mJy per beam area.

might expect recombination to take place after the SN event, with a typical recombination time of $\sim 3,000$ yr. However, the post-SN X-ray flux might be sufficient to significantly delay recombination. The stability of such a structure over the main-sequence lifetime of the ionizing star must be considered. A gas column originally in equilibrium with the surrounding interstellar medium in its cool neutral state could be expected to disperse when ionized on a time scale of $\sim 10^5$ yr.

We have also considered the possibility that the structure might have been shock-ionized following a breakout of the blast wave at a weak point on the shock boundary of the SNR. Such a model¹² has been advanced to explain the filaments which extend far beyond the apparent boundary of IC443. However, the shock would have to advance within the column at a mean velocity of at least four times the velocity of the main remnant shock, and this we view as unlikely.

Features associated with at least five other SNRs have been described as jets. On the smallest scale are the oppositely directed precessing radio jets associated with SS433 within the remnant W50. The radio emission is variable and extends ~ 0.03 pc from the central source in directions aligned with lobes of W50 (ref. 13). It is $\sim 10\%$ linearly polarized and has a spectral index near -0.6 .

A limb-brightened optical protrusion extending about 1 pc from the northern edge of the Crab nebula¹⁴ is most apparent in O III emission¹⁵. A radio counterpart has recently been detected¹⁶ at a level below 1% of the peak brightness of the nebula. This very weak radio feature is highly polarized and is thought to be non-thermal.

On a larger scale, two other remnants, CTB 37A (ref. 17) and CTB 80 (ref. 18), display radio features which we tentatively describe as jets. In the case of CTB 80, arc-like emission can be traced from a central bright core to an estimated distance of 30 pc from the remnant. In CTB 37A, the extended feature is aligned at right angles to the bright shell of the remnant. It is detached from the remnant proper and has the appearance of a misplaced section of shell. In both cases, the spectrum of the emission is distinctly non-thermal with an index not greatly different to that of the SNR.

None of these four jets closely resembles the feature near G332.4.

However, there is a feature associated with the SNR G5.3-1.0 (Milne 56)^{19,20}, which is similar in appearance to the jet and plume described here. The G5.3 jet appears to extend from a compact object out to a distance of about one source diameter, where it fans out into an immense plume. The jet shows no polarization. The plume in G5.3 is discernible to a much greater relative distance than the plume associated with G332.4, which is lost in the nearby confusion. We believe that the two objects fall in the same (SNR) category, and that, contrary to the suggestion of Helfand and Becker²¹, there is no need to invoke a new class of radio source.

Further high-resolution observations of the continuum polarization and of possible recombination line emission for the jet and plume apparently emanating from G332.4+0.1 are required to determine the emission process. New attempts to detect low-level X-ray emission, from either the jet or the postulated central source, would also be valuable.

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Spottiness in the large-scale structure of the microwave background

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Barrow *et al.*¹ have recently considered the large-scale temperature variation ($\Delta T/T$) distribution of the microwave background in the open universe. Interest in this topic has been stimulated by new observations from balloons and the satellite Prognoz. Barrow *et al.*¹ seem to have misunderstood the theory that we have developed²⁻⁴ and arrived at conclusions directly contradicting our results. Here we argue that the main conclusions reached by Barrow *et al.*¹—that concerning the relationship between inhomogeneous and the most general homogeneous anisotropic cosmologies, and their other claim that the sky $\Delta T/T$ pattern of infinite-wavelength inhomogeneities is a superposition of Bianchi type VII models—are erroneous.

The angular pattern of $\Delta T/T$ over the celestial sphere has been called a 'spotty structure' due to the fact that the general form of the perturbation can be represented as a kind of Fourier integral. The basic functions of this expansion are akin to plane waves, and each of them produces a 'spot' (an annulus) in the $\Delta T/T$ pattern of angular size $\theta \sim \Omega = \rho/\rho_{cr}$ which is determined by the space curvature of the universe at the time of observation² (ρ is the total matter density). (In a spatially flat universe ($\Omega = 1$), the 'spot' is a quadrupole structure.) An important feature of ref. 2, distinguishing it from those discussed in the 1950s and 1960s⁵⁻⁷, is its close relationship to the geometry of homogeneous Bianchi type VII and V models. In this sense, the theory from ref. 2 is a generalization of the spot effect⁸⁻¹⁰ inherent in the Bianchi models mentioned above.

Arbitrary inhomogeneous transverse perturbations, which do not perturb the matter density, are directly related to the homogeneous anisotropic models. For these perturbations, one can always choose plane waves that are circularly polarized and consequently, permit the type VII group of motions. In this way, "... arbitrary vortex and gravitational-wave perturbations can be Fourier expanded into the homogeneous modes of type VII ...".² So, the celestial $\Delta T/T$ pattern of transverse perturbations of any scale is a linear superposition of Bianchi type VII spots. Just the opposite occurs with scalar (density) perturbations, the situation Barrow *et al.*¹ deal with.

Scalar waves are nonhomogeneous due to their longitudinal nature. That is why it is, in principle, not possible to relate density perturbations (even of large scale) to homogeneous models of any kind. This directly contradicts the claim by Barrow *et al.* that "... it is possible to model inhomogeneous universes close to the Friedman model as superpositions of the most general homogeneous Bianchi models ...". Nevertheless, the sky pattern of arbitrary scalar inhomogeneities is again a superposition of spots (but nonhomogeneous ones)²⁻⁴. The structure of these nonhomogeneous spots differs from the structure of spots in type VII models.

The angular size of a spot produced by a single scalar plane wave depends on the background space curvature only, and is independent of the perturbation scale, cosmological evolution, recombination dynamics, reheating, missing mass parameters and so on. (These factors determine the fine angular structure and the amplitude of the spot.) The large-scale shape of any spot is unique for all plane waves and can be easily derived using Lobachevski three-geometry (the space geometry of the open universe)^{4,10,11}:

$$\left| \frac{\Delta T}{T} \right| \sim \left(\frac{2\pi \tan \theta/2}{1 + (\pi \tan \theta/2)^2} \right)^2 \quad (1)$$

Here θ is the angle between the wave-vector and the line-of-sight, $\pi = \exp(-h\chi_r)$, h^{-1} is the curvature radius of the Lobachevski space, $\chi_r = \int_{t_0}^t dt/a$ is the geodesic three-distance of the observer (t_0) from the recombination sphere (t_r), $a = a(t)$ is the scale factor. For the euclidian space ($h = 0$) and equation (1) reduces to quadrupole dependence ($\sim \sin^2 \theta$). For the Lobachevski space $\pi < 1$ and all $\Delta T/T$ anisotropy develops inside the annulus of angular radius $\theta_0 = 2 \arctan \pi$ and width $\sim \theta_0$. For small Ω , $\pi \approx \Omega/4$ and the angular size of the spot is Ω . The large-scale profile, equation (1), of a single spot may already serve as an illustration of the effect of spottiness in open universes: for $\Omega = 1$ ($h = 0$) an arbitrary superposition of spots always results in a quadrupole distribution, while for $\Omega < 1$ ($h \neq 0$) a statistical distribution of $\Delta T/T$ with an angular correlation scale $\sim \Omega$ takes place. We now consider the fine structure of non-homogeneous spots.

The value of the wave-vector modulus k determines the fine structure of a spot, which actually consists of a number of narrow concentric rings packed inside the spot annulus. For $k > h$, the large-scale spot profile, equation (1), modulates the sequence of these rings. With decreasing k the rings inside the annulus smear out (the width of every ring increases) and the overall spot profile approaches equation (1) as $k \rightarrow 0$. The spot amplitude can be of any sign, so cold- and hotspots have equal probabilities.

In the infinite-wavelength limit ($k = 0$), plane waves do not perturb space curvature and become homogeneous—they exhibit the translational symmetry of type V models. [This is valid for all plane waves (not only for scalar ones). Recall that for the limit $k \rightarrow 0$, Bianchi type VII group of motions becomes type V. From the physical point of view, each of these models consists of three plane waves on the Friedman background with the wave vectors in the same direction: the scalar one with the infinite wavelength plus the vortex and the gravitational ones. The only difference between the two models is that in the type VII model the latter two waves have finite wavelengths (and are circularly polarized), while in the type V model they are infinite¹¹.] Thus, as was formulated earlier in refs 2-4, any infinite-scale perturbation is a superposition of type V models. This contradicts another conclusion of Barrow *et al.*¹ that infinite-wavelength scalar inhomogeneities are the superpositions of type VII models. The difference in conclusions means that the true cause of disagreement stems from the initial basic assumptions.

So, to analyse nonhomogeneous perturbations, we incorporate the Fourier expansion into the plane waves (which reduces to the well-known Fourier integral over e^{ikx} in the euclidian universe). Barrow *et al.*, in contrast, use the method of covering (piecewise smooth approximation) a nonhomogeneous solution with pieces (patches) of homogeneous models. They have developed their conception on the basis of an evolution formula for the density perturbations $\delta\rho/\rho$ in a dust-like medium (see their equations (16) and (17)). Such perturbations evolve in the same way for all wavelengths. Analogous expressions (with arbitrary functions of spatial coordinates replaced by constants) also exist in homogeneous models (see their equations (6) and (15)) which according to Barrow *et al.* enables one to approximate large-scale density perturbations in its most general form by "... an ensemble of homogeneous Bianchi VII models of differing density ...", that is with varying constants. Such an approximation over the entire spectrum is completely unjustified because homogeneous density perturbations originate from scalar waves with infinite wavelengths. At the same time, to invoke vortex and gravitational-wave modes (which perturb the curvature in type VII models) to calculate the shear evolution (see their equations (6) and (15)) is physically meaningless because we consider the density perturbations only. On the other hand, from the full Fourier integral, one infers that, when covering a large-scale density inhomogeneity with homogeneous patches of finite sizes, the dominant contribution will be due to the essentially nonhomogeneous modes with wavelengths of the order of the inhomogeneity size. The approximation of a nonhomogeneous solution with homogeneous ones is justified in the limit of infinite-wavelength perturbations only, but then it requires only type V models.

The analogy in the behaviour of homogeneous and nonhomogeneous scalar perturbations, based on the invariant evolution law for $\delta\rho/\rho$, cannot be extended to non-dust-like media, while the effect of spottiness is always there. It results from the global curvature of the universe ($\Omega < 1$)^{2,8} and depends only quantitatively on details of evolution.

We conclude by emphasizing that in the most general case, the spotty structure of $\Delta T/T$ in open universes is described by a Fourier integral and cannot, in principle, be reduced to an expansion in homogeneous Bianchi models. The discovery of a 'spotty' structure (or of its absence) of the microwave background would be extremely important for cosmology. It could answer basic cosmological questions such as what was the spectrum of primordial perturbations and what is the total density of all kinds of cosmic matter (including its invisible components).

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John D. Barrow*, R. Juszkiewicz† & D. H. Sonoda* reply

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Lukash and Novikov have given an interesting survey of their analyses of some of the features that may exist in the microwave background radiation in open universes. Here, we answer some criticisms that they make of our earlier analysis¹, and point out a further unusual feature that appears in the temperature distribution of the most general flat and open cosmological models.

Lukash and Novikov claim that in ref. 1 we erroneously represented infinite wavelength density perturbations by ensembles of Bianchi type VII universes at linear order, rather than by cosmological models of Bianchi types I or V, as required. This claim is incorrect. We found, and stated, that infinite-wavelength, pressure-free density inhomogeneities could be represented by ensembles of VII models only when one of their defining parameters, $x = h^{1/2}(1 - \Omega_0)^{-1/2}$, tends to infinity. In this limit, the type VII_h and VII₀ models specialize identically to the type V and I models respectively; here, Ω_0 is the density of the Universe today in units of the critical density and x or h is an arbitrary constant once Ω_0 is specified. (In the VII₀ case, the parameter x still exists when the limits $h \rightarrow 0$ and $\Omega_0 \rightarrow 1$ are taken simultaneously; see ref. 2 for details). Accordingly, all computations in ref. 1 of the effects of inhomogeneity on the background radiation were performed using the type I and V models, as Lukash and Novikov argue is necessary.

Finally, we would like to comment on one important difference between the hotspot patterns that arise in open universes of type VII and those arising in open universes of type V which were described by Lukash and Novikov and by ourselves¹. Although the type VII models are not relevant for the description of density inhomogeneities, they can describe the effects of gravitational waves and vorticity on the temperature anisotropy of the microwave background.

The general equation used by Lukash and Novikov to describe the hotspot structure is based on the type V paradigm where the spatial curvature is isotropic, and does not completely describe the most general hotspots generated by vortical and gravitational wave distortions; these will be described by the type VII_h behaviour rather than the simpler type V case. The geodesics in open type V universes exhibit focusing of a quadrupole temperature profile into a hotspot feature but with no change in the azimuthal coordinate of the geodesics propagating from their last scattering to the observer today. However, in type VII models there arises a geodesic spiralling effect which is superimposed on the hotspot structure. This feature was first noted by Collins and Hawking³ and its observational effects are examined in detail in ref. 2. Figure 1 shows examples of the temperature anisotropy patterns predicted in VII₀ and VII_h universes. In the VII₀ case, where $\Omega_0 = 1$, there is a pure quadrupole plus the geodesic spiralling effect, whereas in an open VII_h model with $\Omega_0 = 0.7$, we see a spiral plus quadrupole pattern focused into a spiral hotspot. The number of turns of the spiral is inversely proportional to the parameter x . In the limit $x \rightarrow \infty$, in which case the spatial curvature becomes isotropic, the VII₀ and VII_h models become those of types I and V again, the spiral effect disappears and we recover either a quadrupole or the

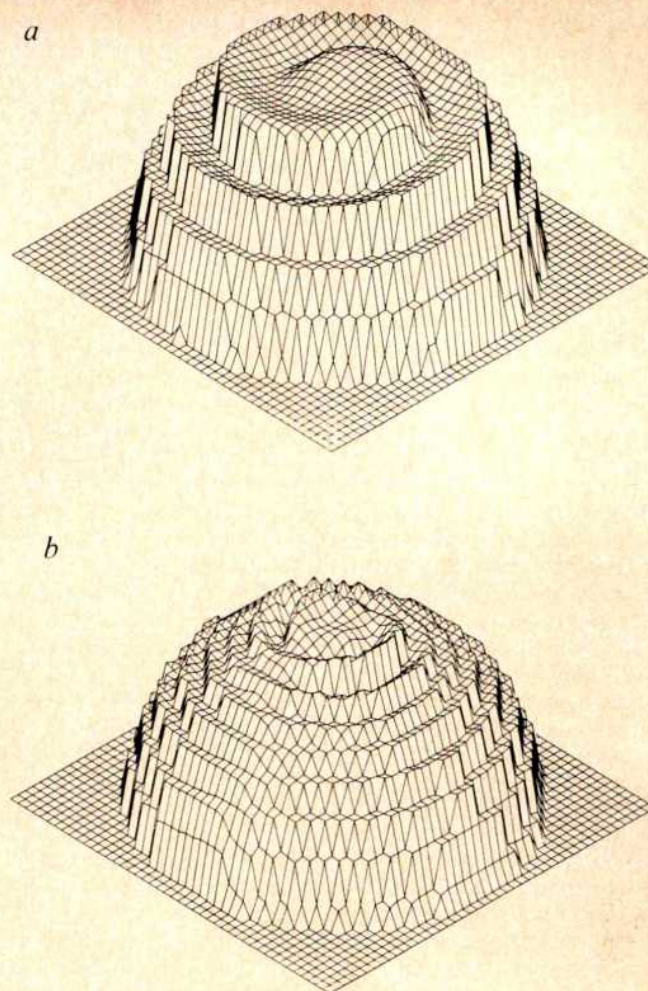


Fig. 1 Microwave background temperature profiles, $T(\theta_0, \phi_0)$, predicted in the region of observed angles $0 < \phi_0 < 2\pi$, $\pi/2 \leq \theta_0 < \pi$. The radial distance represents the magnitude of $T(\theta_0, \phi_0)$. We assume that the last scattering of the photons occurred at a redshift of 1,000, and the anisotropy amplitude is taken to be the maximum allowed by current observations. *a*, Spatially homogeneous-type VII₀ cosmological model with $x=0.067$ and $\Omega_0=1$. *b*, An open spatially homogeneous cosmological model of type VII_h with $x=0.067$ and $\Omega_0=0.7$. In *a*, the pattern is approximately a pure quadrupole modulated by the geodesic spiralling effect. In *b*, both the quadrupole and spiral patterns seen in *a* have been focused into a region of smaller angular scale towards the axis $\theta_0 = \pi$ by the negative spatial curvature. The result is a spiral hotspot. The spirals are left-handed in the observed angles. As $x \rightarrow \infty$ the spiral effect disappears and the hotspot in *b* degenerates into the simple variety found in the type V models, and *a* becomes the pure quadrupole of type I. For further details and discussion see ref. 2.

simple hotspot structure discussed in the preceding letter and ref. 1. Note that because this spiral feature does not arise in closed universes, it may enable observers to determine whether the Universe is open or closed, no matter how close its density lies to the critical value².

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A search for evidence of nuclearites in astrophysical pulse experiments

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De Rújula and Glashow¹ have suggested that nuclearites, aggregates of up, down and strange quarks in roughly equal proportions, may form a component of the material reaching the Earth from the Galaxy. On traversing the atmosphere they will look something like meteors, but will travel faster. The masses of these aggregates may vary over a wide range and their velocities will be typically 250 km s^{-1} , corresponding to the Sun's galactic rotation. If all the dark matter in the Galaxy is assumed to be in the form of nuclearites, a limit can be set to the incoming flux. We report here upper limits derived from four experiments, originally carried out to detect cosmic γ rays, as well as some derived by other authors, which would be sensitive to pulses of light from nuclearites in the lower atmosphere. Our upper limits are compatible with the suggested maximum flux and competitive with a search for tracks etched in mica², which could also be caused by nuclearites.

De Rújula and Glashow show that the visual magnitude of an atmospheric nuclearite:

$$M = 10.8 - 1.67 \log_{10} (m/10^{-6}) + 5 \log_{10} (h/10 \text{ km}) \quad (1)$$

where m is the mass of the nuclearite in grams and h its distance from the detector. The nuclearite will radiate predominantly in the lower atmosphere, effectively below:

$$h_{\text{max}} = 2.7 \text{ km} \ln (m/1.2 \times 10^{-5} \text{ g}) \quad (2)$$

In our experiments, m ranged from 10^{-3} to 10 g, and h_{max} was typically 20–30 km, except in one case where it was constrained by the arrangement of the optical system.

The first five experiments that we consider were carried out at the Whipple Observatory, Arizona. The first³, in 1969–72, used the 10-m reflector to search for γ rays from the Crab Nebula or, for pulsars in the 10^{11} -eV region. A total of 112 h of observation were taken at various zenith angles. To trigger the system, ~ 50 photoelectrons were required in an integration time of 10^{-8} s. In a subsequent analysis⁴, a search was made in the data for bursts of events lasting up to 0.1 s. This analysis is the basis of the present discussion; 12 events were required to constitute a burst. The transit time through the field at a typical detectable height was $\sim 10^{-2}$ s. To produce 12 events from random fluctuations in 10^{-2} s, with a basic integration time of 10^{-8} s, requires about a 4.5σ upward deviation from the mean light of the nuclearite in these 10^{-8} s intervals. (The night-sky background contribution⁵ was small, $\sim 10\%$ of that from the nuclearite). Fifty photoelectrons are needed, σ therefore is 11.1, and $\sigma^2 = 123$, the mean number of photoelectrons per 10^{-8} s interval. With a 15% photocathode efficiency at the photomulti-

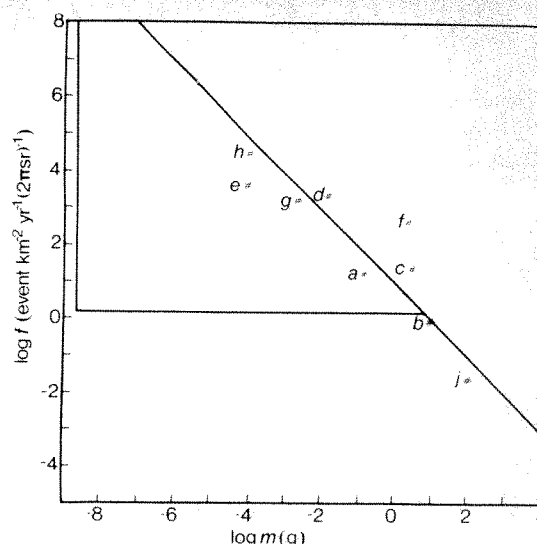


Fig. 1 Mass of the nuclearites plotted against incoming flux. The sources are detailed in Table 1.

plier, we obtain a photon number of 823 photons in 10^{-8} s average illumination over the 10^{-2} s transit time. The area of the reflector was effectively 60 m^2 , leading to a flux of 1.37×10^{-3} photons cm^{-2} in 10^{-8} s, or 1.37×10^5 photons $\text{cm}^{-2} \text{ s}^{-1}$. Converting this to $\text{erg cm}^{-2} \text{ s}^{-1}$ to obtain the visual magnitude⁶ and taking $0 \text{ mag} = 8.5 \times 10^{-6} \text{ erg cm}^{-2} \text{ s}^{-1}$, we obtain a limiting magnitude of 3. Combining equations (1) and (2) the mass became 0.16 g , h_{max} was 25 km , and a typical distance h was 15 km .

The surface area of the conical frustum from 2.2 km (altitude of observatory) up to 25 km at a mean zenith angle of 25° and full field of view 1° was 16.6 km^2 . Upward moving nuclearites occur for this mass, so the whole area is effective. The statistical upper limit, at the 99% level, from the burst analysis, was $477 \text{ events yr}^{-1}$. We therefore obtain an upper limit flux of $28.7 \text{ events km}^{-2} \text{ yr}^{-1} (2\pi \text{ sr})^{-1}$, at a mass $m = 0.16 \text{ g}$. This limit is plotted as a in Fig. 1, the diagonal line is the upper limit to the cosmic flux, derived by De Rújula and Glashow on the assumption that the entire dark matter of the Galaxy consists of nuclearites. The triangular area in Fig. 1 represents the limits derived in ref. 1 from an experiment by Price *et al.*² using the etching of tracks in buried mica samples, originally intended for the detection of monopoles. It will be seen that our limit falls below the maximal flux and is compatible with the mica experiment.

We are aware of eight other experiments which can be similarly analysed. Their main features are summarized in Table 1 and the resultant flux limits plotted in Fig. 1.

The results are generally comparable with those obtained in the mica experiment. Only that of Ogelman¹⁴ can confidently be placed below both the maximal flux and the mica limit. Experiments designed specifically for nuclearite detection could, however, significantly reduce the limits by optical techniques.

Table 1 Cosmic flux limits for nuclearites of various masses

Ref.	Mirror diameter (m)	Mass (g)	Flux limit (events $\text{km}^{-2} \text{ yr}^{-1} (2\pi \text{ sr})^{-1}$)	Point in Fig. 1
7	1.5×2	10	1.15	b
8	1.5×3	3	4.3	c
9	10×1	1.5×10^{-2}	1,700	d
10	10×1	10^{-4}	5,000	e
11	1.5×1	0.2	380	f
12	0.8×1	3×10^{-3}	1,710	g
13	1×1	10^{-4}	3.5×10^4	h
14	None, PM tubes only	250	0.013	j

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Penrose tiling observed in a quasi-crystal

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Three-dimensional icosahedral symmetry has been reported¹ for an alloy consisting of aluminium with 14 atomic% manganese. The electron diffraction patterns appear consistent with a three-dimensional Penrose tiling² or quasi-lattice^{2,3}, although one report has suggested a 20-fold multiple twinning of a close-packed structure⁴. Clearly, however, the determination of the reciprocal lattice is only a first step; it is a further problem to determine the atomic motif(s) required to complete the structure. High-resolution electron microscope images of this alloy, reported here, reveal the localized pentagonal pseudo-symmetry of the structure and show that it projects as a two-dimensional Penrose tiling^{2,5}. A structural model is derived, based directly on the experimental images and an analysis of the polyhedra occurring in the crystallographic phase Al_6Mn .

The specimen was provided through the courtesy of Dr Leo Bendersky (National Bureau of Standards, Washington, DC) and was prepared as described in ref. 1. Figure 1 shows a view, looking exactly along one of the 5-fold symmetry axes, of an area considerably less than 100 Å thick. The imaging conditions in the JEOL-200CX instrument used were chosen so that, according to the usual theories of image contrast⁶, we would expect the areas of black contrast to represent regions of relatively high atomic density, at a resolution of ~2.4 Å. There is a striking resemblance to the original Penrose drawings of tiling patterns⁷. The enlargement in Fig. 2a shows a pronounced localized 5-fold pseudo-symmetry, the centre of which maps almost perfectly onto one of Penrose's drawings (Fig. 2b). In general, the pentagonal symmetry is short-range, rarely extending further than 15 Å, as expected for Penrose tiling. Rotation of the electron micrograph with respect to a fixed glancing angle reveals five interpenetrating sets of spacings in the ratio 1:τ ($=1+\sqrt{5}/2$), characteristic of the Fibonacci series. Note also the decagonal rings (diameter 14.5 Å) of white spots, pentagonal stars of white spots (separation 5.4 Å) and the decagonal and pentagonal rings of black contrast (diameters 8.8 and 5.4 Å, respectively). These are shown schematically in Fig. 2c, which also allows the five Fibonacci sequences comprising this area to be identified.

Following the idea of Mackay², we tried to build a structural model using acute and obtuse rhombohedral units, taking the rhombic edge to be 2.7 Å (the mean Al-Al separation in crystalline aluminium). Although some features of the image could be matched, for example the decagonal rings of black contrast correspond geometrically to the projected waist of Mackay's rhombic triacontahedra, we could not match the diameters or disposition of the small dark rings on either the larger decagonal rings of white spots or the pentagonal stars of white spots. We conclude that the structure is not based on such slightly distorted elements of cubic close-packing, at least if the white spots represent relatively low densities of atoms. It appears that, in this case, we must search for a different structural motif for the quasi-lattice.

A careful consideration of the structure reported⁷ for Al_6Mn reveals (Fig. 3a) that it may be considered as a stacking of two symmetrically equivalent layers composed of polyhedra which differ from an icosahedron only in that one vertex is unoccupied (Fig. 3a inset). These truncated icosahedra (or TICOS) share edges within layers (Fig. 3b) and corners between layers (Fig. 3c). Note that each TICOS contains: one Mn atom not shared with adjacent polyhedra; eight Al atoms, shared between those TICOS within layers; two Al atoms, shared with one other

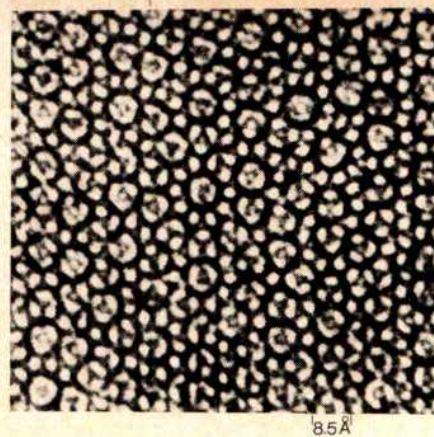


Fig. 1 High-resolution electron microscope image of Al:14 at.% Mn viewed along a 5-fold symmetry axis. Note the localized centres of pentagonal symmetry and absence of translational symmetry. Large-scale and fine-scale Penrose tiling patterns may be traced over.

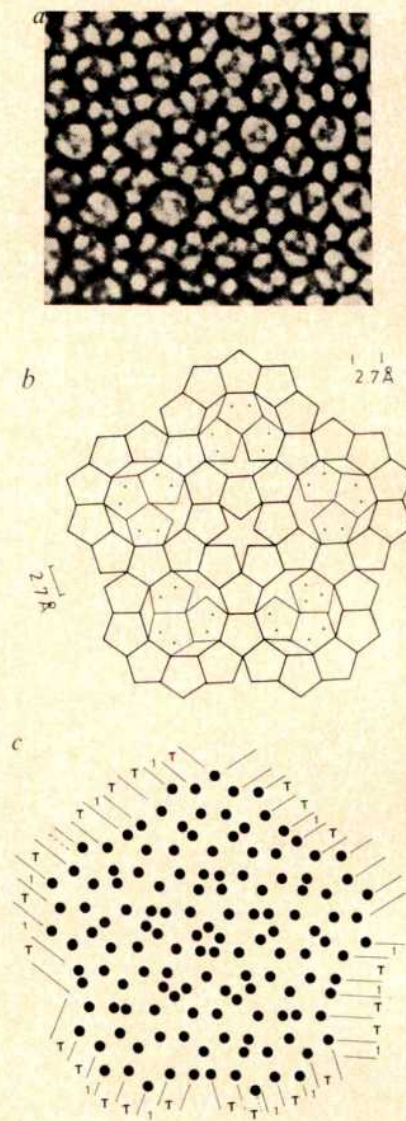


Fig. 2 Enlargement of a pronounced centre of pentagonal pseudo-symmetry (a), together with a copy of an original Penrose drawing, showing almost perfect mapping of white spots (pentagon centres) and dark contrast (lines) (b). c, Schematic drawing showing decagonal and pentagonal rings representing nodes of the Penrose tiling pattern, allowing the interpenetrating Fibonacci series of spacings to be identified.

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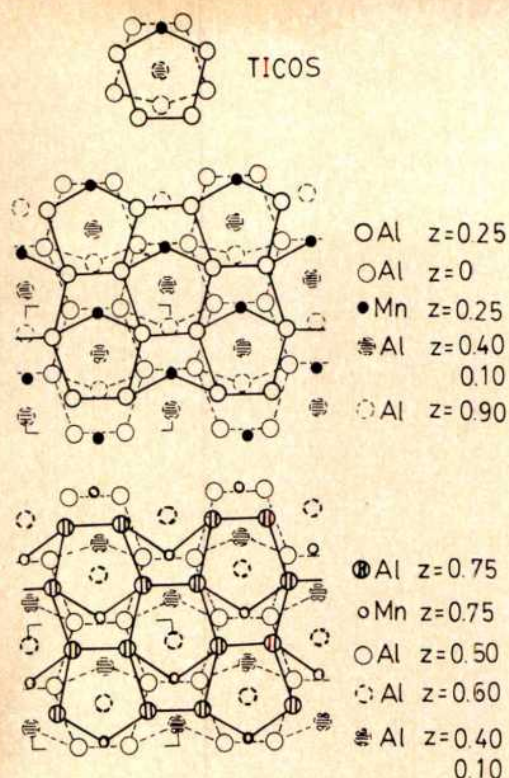


Fig. 3 [001] Projection of crystalline Al_6Mn , showing edge-sharing of truncated icosahedra (TICOS) within layers and corner-sharing between layers. Each TICOS contains one Mn atom (on surface) and one Al atom (internal) which are not shared with adjacent TICOS, the eight remaining Al atoms being shared between two TICOS.

TICOS in adjoining layers; and a further Al atom inside each TICOS, which is therefore unshared. The net result is, of course, a stoichiometry of Al_6Mn .

This analysis suggested that the observed Penrose tiling could be explained by a cooperative rearrangement of TICOS. Figure 4 shows two levels of a model for the central area of Fig. 2. The pentagonal star at the first level is surrounded by decagonal rings composed of rings of five TICOS at both first and second levels, tessellated according to the observed tiling pattern. The model could readily be extended, at least in two dimensions, to match details of the dark (and white) contrast variations in the experimental images. Note that the algorithm used was that a white spot represents the centre of a column of TICOS along the projection axis. Also, there is a relatively low density of Al atoms inside the TICOS, Mn always occupying asymmetrically coordinated sites on the pentagonal rings at the surface of individual TICOS. The stoichiometry Al_6Mn is preserved in this model, since on average each TICOS retains the same number of shared Al atoms (8) and unshared Mn (1) and Al (1) atoms.

The essential icosahedral geometry of the TICOS gives us some confidence, justified so far by model-building, that a fully three-dimensional model may be achieved in which six such interpenetrating two-dimensional networks coexist. Dark-field observations suggest correlation lengths of ~ 50 – 100 Å along the 5-fold axes. Consideration of the configurations of black and white contrasts expected for overlapping in projections of the possible two-dimensional patterns has so far been consistent with the experimental images. We are now investigating whether existing electron dynamical techniques can be used to calculate the images, and it should be possible to calculate images for appropriate sections of the model using existing techniques for non-periodic objects⁸.

The experimental observations have already been extended to both 3-fold and 2-fold axes. Pseudo-symmetric centres are again observed. In addition, stacking faults and other defects occur in the quasi-lattice (sight along the dark fringes in Fig. 1);

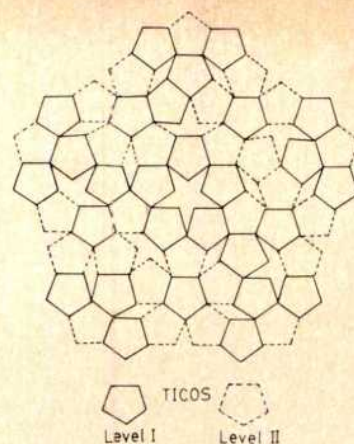


Fig. 4 Assemblage of TICOS polyhedra to generate two-dimensional Penrose tiling reproducing the central area of Fig. 2. TICOS were placed at the centre of each white spot in the experimental image.

localized areas appear to transform to less-ordered (glassy) states and the crystalline phase may also occur (L.A.B. and P.J.L., in preparation).

The structural principles described above, which we believe to be justified reasonably well by both the experimental images, and our analysis of the structure reported for Al_6Mn , should be regarded as tentative. Nevertheless, we hope they will provide a realistic basis for future studies of the alloy's structural and physical properties. What is most intriguing, of course, is whether we are concerned with a material having singular structural properties because of the chemistry of Al and Mn, or whether the principles suggested by the quasi-crystal concept will find more widespread application. We favour the latter possibility.

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Quantization effects in the photoelectrochemistry of superlattice photoelectrodes

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Size quantization effects have created much recent interest. In solid state physics, for example, the novel effects exhibited by thin layered semiconductor structures in the form of multiple-quantum-wells (MQW) or superlattices (SL) are under intense study^{1–7}. In photoelectrochemistry, quantum-size effects produced by very small (<100 Å) semiconductor particles have been analysed^{8–11}. We report here the first quantum size effects produced in a photoelectrochemical system using a superlattice photoelectrode. The electrode contained 40 alternating layers of undoped GaAs and $\text{GaAs}_{0.5}\text{P}_{0.5}$ each 250 Å thick. This structure produces a strained-layer superlattice (SLS) with 20 GaAs quantum wells having a well width of 250 Å; the $\text{GaAs}_{0.5}\text{P}_{0.5}$ barrier widths are also 250 Å thick. The barrier heights for electrons in the conduction band

and for holes in the valence band for this SLS are 0.28 eV and 0.30 eV, respectively⁶. The photocurrent-wavelength response shows remarkable structure at room temperature that arises from the presence of discrete energy levels in the GaAs quantum wells. This structure is readily resolvable into multiple photocurrent peaks that are in excellent agreement with theoretical predictions of the discrete energy levels in the quantum wells. These results have important implications for photoelectrochemical energy conversion and for the characterization and analysis of SL and MQW structures in general.

The SLS structure was grown by metal-organic chemical vapour deposition (MOCVD) using continuous flows of trimethylgallium, PH_3 , and AsH_3 . The growth rate and growth temperature were $0.1 \mu\text{m min}^{-1}$ and 750°C , respectively. A $10\text{-}\mu\text{m}$ $\text{GaAs}_{1-x}\text{P}_x$ buffer layer graded from $x=0$ to 0.25 in five equal steps was inserted between the $\text{p}^+\text{-GaAs}$ substrate and the SLS structure. The $\text{p}^+\text{-GaAs}$ wafers were oriented 2° off (100) towards (110) and were cleaned before growth using a 6:1:1 $\text{H}_2\text{SO}_4:\text{H}_2\text{O}_2:\text{H}_2\text{O}$ etch. The SLS layers are not lattice-matched and large strains are, therefore, generated in the layers both parallel (biaxial shear strain) and perpendicular (hydrostatic strain) to the layers^{5,6}. A scanning electron micrograph (SEM) of the layered structure is shown in Fig. 1; the large strain results in wavy, but equidistant, layers. The top layer is GaAs. The SLS structure studied in this work has the same configuration as that reported by Gourley and Biefeld⁶ to exhibit quantization effects in optical and photoluminescence data.

The theoretical energy-level scheme for a $250 \text{ \AA}$ $\text{GaAs}/\text{GaAs}_{0.5}\text{P}_{0.5}$ SLS is shown in Fig. 2. The energy-level calculation is a simple modification of that previously reported⁶ and includes the effects of lattice strain. The selection rules for optical transitions in quantum wells require that $\Delta n=0$, where n is the quantum number of the respective valence and conduction band states. The allowed transitions are indicated in Fig. 2.

Photoelectrodes were prepared from the SLS wafer by contacting the $\text{p}^+\text{-GaAs}$ substrate with a gold ohmic contact and sealing all but the front SLS surface with epoxy; the electrodes were then immersed in an electrolytic cell containing either 0.2 M ferricyanide in 1 M H_2SO_4 or 0.1 M Eu^{3+} in 1 M HClO_4 .

Photocurrent action spectra (quantum yield plotted against wavelength) at room temperature are shown in Fig. 3 for two different samples of the original SLS wafer in the $\text{Eu}^{3+}/\text{HClO}_4$ electrolyte; the electrode potential was held at -0.8 V (versus SCE) and the photocurrents were cathodic. A remarkable feature is the clear presence of photocurrent peaks in the spectra; conventional GaAs photoelectrodes in the form of single crystals or thick single layers deposited by MOCVD were examined and showed the usual photocurrent onset at the band edge of GaAs followed by a smoothly increasing monotonic photocurrent that saturates to a constant value at $\sim 875 \text{ nm}$. The experimental data for the superlattice photoelectrode could be fitted extremely well by a sum of seven gaussian peaks constrained to a common

linewidth. The peak positions and heights are indicated in Fig. 3. The data could be fitted equally well using an eV scale rather than wavelength. In the former case, the fitted linewidth value was 50.6 meV.

The data show that the peak positions for photoelectrodes prepared from different regions of the SLS wafer are the same, while the peak intensities vary. We believe that the various peak intensities reflect electron transfer into the electrolyte from the discrete levels in the quantum wells. If the electrons were being injected into the electrolyte only from the lowest quantum level, then the relative peak intensities would be constant for a given quantum well geometry. Also, the observed photocurrent spectra are not consistent with that expected for absorption into the discrete levels³, followed by relaxation to and charge transfer from the lowest state. Thus, Fig. 3 shows that the SL layers are of uniform thickness, but the electron transfer probability from the discrete levels vary with position in the sample. This result is consistent with the SEM pictures (Fig. 1) showing uniform layer thicknesses but irregular outer surface layers; the outer surface in contact with the electrolyte will control the electron transfer kinetics.

Support for electron transfer from the higher lying states in the quantum well comes from the observation that the higher energy photocurrent peaks ($680 < \lambda < 750 \text{ nm}$) show markedly lower relative intensities compared with the lower-energy photocurrent peaks when a lower lying (more positive in the SCE scale) redox couple, such as ferricyanide, is used instead of Eu^{3+} . This effect is attributed to the poorer overlap of the higher lying levels in the conduction band quantum well with the energy levels in the electrolyte.

The experimental photocurrent spectra agree very well with the first six allowed ($\Delta n=0$) transitions shown in Fig. 2. The seventh experimental peak at 1.77 eV fits to a non-allowed, $\Delta n=1$ transition from the $n=7$ level in the valence band to the $n=6$ level in the conduction band; this is labelled transition 6' in Fig. 3. Non-allowed, $\Delta n=1$ transitions have also been seen in SLS optical data⁶.

As seen in Fig. 3, the photocurrent response has a maximum at $\sim 750\text{--}780 \text{ nm}$, and then falls to nearly zero at wavelengths $< 680 \text{ nm}$; in this sample the response remained near zero down to 400 nm . This drop-off in photocurrent below 750 nm is, of course, highly detrimental to good solar conversion efficiencies in photoelectrochemical devices. However, we believe this drop-off is not an intrinsic characteristic of superlattice or MQW electrodes; other samples do not show this behaviour. A possible explanation is that the higher energy photons are absorbed closer

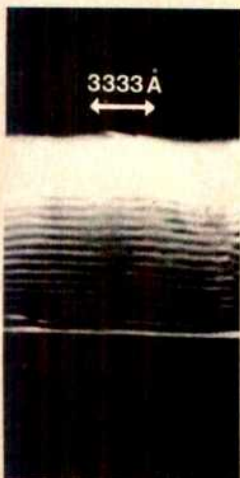


Fig. 1 SEM photograph of SLS cross-section; outer surface is at top. Wavy layers reflect strain from lattice mismatch.

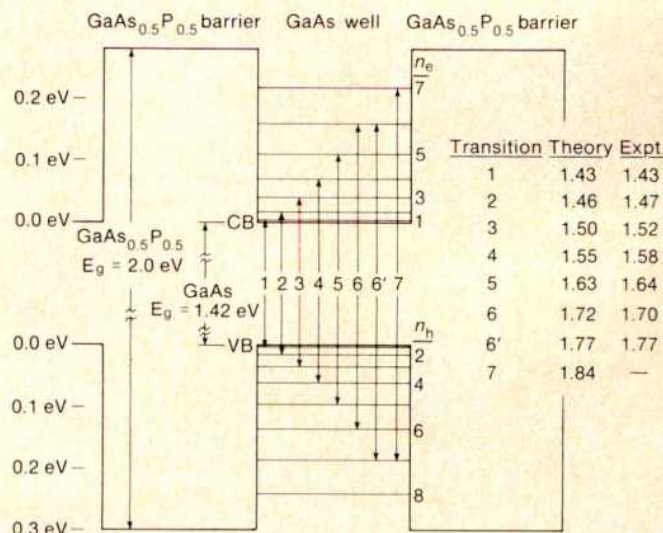


Fig. 2 Theoretical energy level scheme for $\text{GaAs}/\text{GaAs}_{0.5}\text{P}_{0.5}$ strained-layer superlattice (SLS). Allowed transitions ($\Delta n=0$) are shown, along with a non-allowed transition ($\Delta n=1$) from $n_h=7$ to $n_e=6$. The theoretical transition energies are compared with the experimental values. CB, conduction band; VB, valence band.

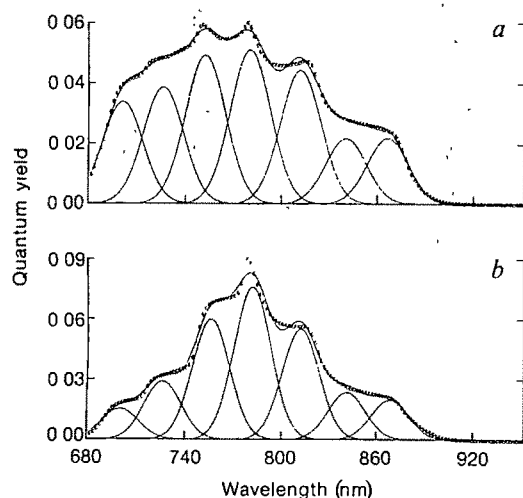


Fig. 3 Photocurrent-wavelength spectra for GaAs/GaAs_{0.5}P_{0.5} SLS. *a* and *b* represent two different regions of the SLS wafer. Solid lines, theoretical fits to seven gaussian lines with equal linewidths

to the outer surface of the SLS, where the layer distortions and strains are greater (see Fig. 1), and hence suffer faster e^-h^+ recombination rates.

The quantum efficiencies exhibited by the present sample are consistent with electron diffusion lengths of the order of 0.05 μm . As expected, this is much lower than the values of 1–2 μm for bulk GaAs or for diffusion parallel to the superlattice layers¹². The lower values for the perpendicular direction are caused by inhibition of charge transfer by the GaAs_{0.5}P_{0.5} barriers. Significant penetration through the relatively thick barriers could be attributed to enhanced tunnelling created by strain-induced defect states in the barrier. Another possibility is that thermionic emission processes may have a role. Further work is required to understand these results more completely.

The good resolution of the photocurrent spectra even at room temperature is surprising; optical absorption and emission experiments are usually done at low temperatures to produce good spectral resolution. Further experiments as a function of SL composition, well and barrier dimensions, and temperature are in progress.

The ability of MQWs and SLs to absorb light at discrete quantum levels and permit charge transfer from these levels provides a potential mechanism for achieving the high theoretical thermodynamic conversion efficiencies predicted for hot carrier injection processes¹³. This is because carrier relaxation to the semiconductor band edges (defined for bulk material), with its attendant thermal loss, is inhibited by the presence of discrete energy levels with relatively large separation.

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Double seismic zone in Tonga

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The clear evidence of a double seismic zone beneath Japan^{1,2} has led seismologists to search for other such zones elsewhere. Because they have been found in few other areas^{3–6}, double seismic zones are thought to be rare and to depend strongly on a peculiar combination of external conditions (such as age of the descending slab, and the convergence rate)⁷. We report here a new double seismic zone which has been found beneath the Tonga arc⁸, where the stress state in the subducting slab has been described as strongly down-dip compressional^{7,9,10}. This discovery suggests that the double seismic zone is a general feature of subducting slabs and may be found in many other subduction zones.

A double seismic zone is a zone of double-planed activity of intermediate depth earthquakes in the subducting lithosphere, usually extending from 60 to 180 km depth (Fig. 1). The two planes are generally separated by $\sim 40 \text{ km}^{1,2,11}$ at the shallow end and gradually merge at depth. The focal mechanisms of earthquakes in the upper and the lower planes tend to have compressional and tensional axes in the direction of the dip of the subducting slab, respectively¹. Double seismic zones have been reported in Tohoku, Japan^{1,2,12,13}, Kuril and Kamchatka^{3,14}, eastern Aleutians^{5,6} and Peru⁴. There have also been suggestions of double seismic zones in the central Aleutians¹⁵ and Marianas¹⁶, but the evidence is not conclusive^{7,17,18}.

Figure 2 shows a view of the general seismicity in the Tonga region with some tectonic features. The deeper portion ($> 300 \text{ km}$) of the slab has a high level of seismic activity and a down-dip compressional stress (or strain) state inferred from the earthquake focal mechanisms^{10,19,20}. This has led to conclusions that the slab cannot penetrate into the lower mantle in this region^{19,20}. It has also been suggested that the stress state of the intermediate depth range of the subducting slab under the Tonga arc is strongly down-dip compressional^{7,9,10}. Isacks and Molnar⁹ state that "a compressional stress is transmitted through nearly all parts of the slab". Even after the discovery of a clear double seismic zone in Japan, neither Billington¹⁰ nor Fujita and Kanamori⁷ hinted at the possibility of a double seismic zone in the Tonga region, although they identified one event which shows down-dip tension at a depth of 186 km. However, a careful examination of the mechanisms and depths of earthquakes reveals that there are several down-dip tensional events in this region. They are located consistently deeper than and seawards of the down-dip compressional events, suggesting the presence of a double seismic zone.

Figure 3 clearly suggests the presence of a double seismic zone from a depth of $\sim 60 \text{ km}$ to $\sim 200 \text{ km}$. This region (azimuth 109° to 116°) was chosen because both the geometry of the subducting plate (inferred from seismicity) and the focal mechanisms suggest that the deformation of the slab occurs in a simple two-dimensional manner⁸. North of azimuth 109° ($\sim 17.5^\circ \text{ S}$), the end of the subduction zone results in hinge faulting, introducing more complicated stress and strain structure in the slab. South of azimuth 116° ($\sim 24.5^\circ \text{ S}$), the lateral bend of the slab near 26° S prevents a simple two-dimensional view of the subduction process.

Cross-sections with narrower azimuth ranges (every 1° along the strike of the trench)⁸ including smaller events show that the down-dip compressional and tensional events are consistently located on the upper and the lower edge of the zone of seismicity, respectively, in every section. The result of relative relocation (Fig. 4) supports the presence of a double seismic zone. The down-dip tensional events in Figs 3 and 4 are consistently located seaward of and below the down-dip compressional events, exactly as expected for a double seismic zone.

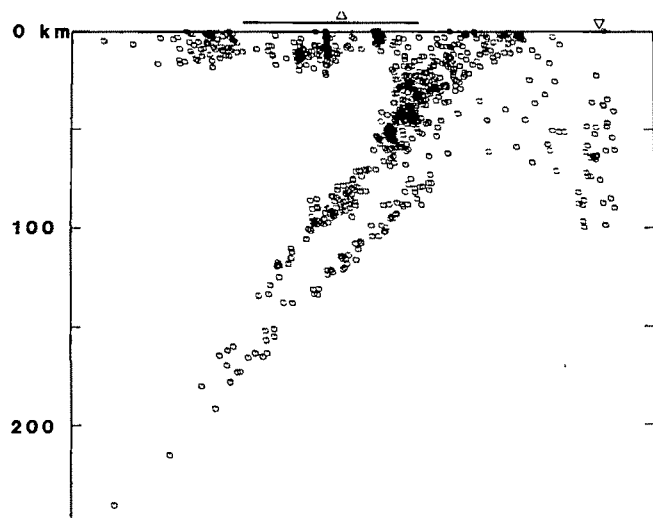


Fig. 1 Double seismic zone beneath the northern Honshu arc, Japan (after ref. 1). The precise determination of the microearthquake hypocentres using data from the seismic network of Tohoku University revealed a clear separation of two seismic zones. The figure is vertically exaggerated by a 2:1 ratio.

What are the implications of this discovery of the double seismic zone beneath the Tonga arc? First, the double seismic zone is a rather persistent feature of subducting slabs. Tonga has the most active deep seismicity but the compressional strain (due either to inability of the slab to penetrate into the lower mantle^{19,20} or to the strong viscous resistance to the subduction in the mesosphere⁹) transmitted from below 200 km depth cannot overprint and erase the pre-existing double seismic zone. On this basis, I predict that there is also a double seismic zone beneath the Izu-Bonin arc, even though it has active deep seismicity and down-dip compressional stresses. Furthermore, I speculate that a double seismic zone will be observed in most subduction zones (at least in those areas where both down-dip compressional and tensional events are already observed^{7,9}). The observation of double seismic zones has been prevented by the lack of high-quality local networks in most subduction zones and the fact that the time span of reliable observations of earthquake focal mechanisms is too short. It has also been difficult to distinguish shallow (<80 km) down-dip tensional events from shallow-angle thrust faulting events, which are very common in subduction zones, without detailed wave-form analysis. This is because they have similar focal mechanisms and the reported depths (obtained by the International Seismology Centre (ISC) or by preliminary determination of epicentre) of shallow events are not reliable. A systematic analysis of these events, which can now be undertaken through the Global Digital Seismograph Network²¹⁻²³, will help to identify more down-dip tensional events.

Second, this discovery constrains possible mechanisms for the origin of the double seismic zone. It excludes geometrical sagging^{24,25} as a possible mechanism since the strong down-dip compressional strain transmitted from below 200 km could easily overprint strain features caused solely by sagging. The simple geometrical unbending^{15,16,26} with a brittle-plastic rheology suggested by Tsukahara²⁶ seems to be the best description of the physical mechanism of the double seismic zone.

Above a depth of ~60 km, oceanic lithosphere bends downwards when it subducts into the mantle. The fact that the subducting lithosphere appears to be straight in the mantle below a depth of ~200 km indicates that the lithosphere must unbend in a depth range from ~60 to ~200 km to become straight below a depth of ~200 km. A simple geometrical argument shows that the strain rate due to this unbending is of the order of 10^{-15} to 10^{-14} s^{-1} above a depth of ~100 km where most of unbending is taking place and is high enough to account for all the energy released by earthquakes of the double seismic zone⁸. Even below a depth of ~100 km where the slab appears to be straight, the

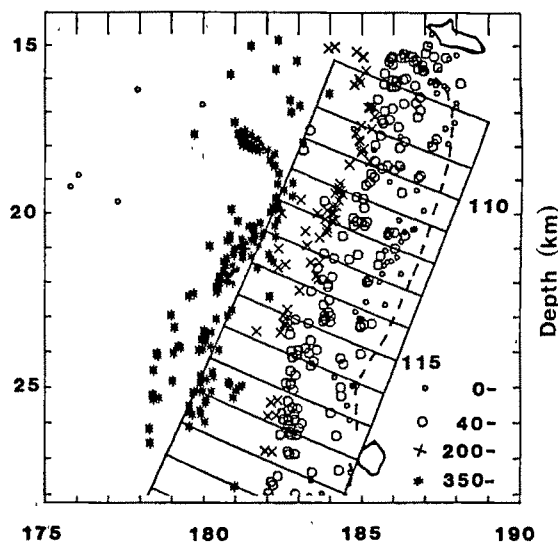


Fig. 2 Schematic view of the general seismicity of the Tonga subduction zone. The depth-constrained ISC events are plotted with tectonic features. The broken line represents the trench. The lines perpendicular to the trench are parts of the great circles which pass through the pole of the arc and the numbers denote the azimuth of the great circle measured clockwise from the north at the pole of the arc. The pole of the arc is located at 20.6° N , 102.8° E , so that the 100-km isodepth contour of Isacks and Barazangi⁴ is 90° distant away from the pole. The two lines parallel to the strike of the trench are at the distances 87.5° and 92.5° .

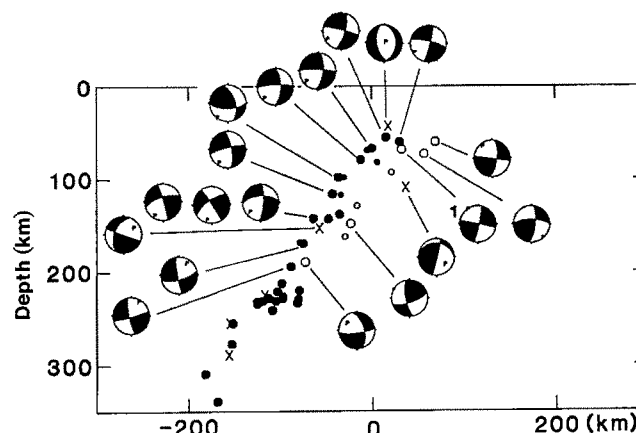


Fig. 3 Cross-section perpendicular to the trench. All the intermediate-depth (60 to 350 km) events occurring between azimuths 109° and 116° for which focal mechanisms are available, are plotted: ●, Down-dip compressional; ○, down-dip tensional events; ×, events which are not either compressional or tensional. The small circles are events for which only the type of the mechanism can be determined⁸. The depths are all constrained by pP-P times reported with ISC. For the down-dip tensional events, depths are also checked by WWSSN seismograms. For events shallower than 200 km, the near side of the focal sphere is also plotted. Event 1: the depth (61 km)²³ of this large ($M_0 = 1.4 \times 10^{28} \text{ dyn cm}$)²³ normal fault event (22 June, 1977) is controversial²³. The aftershock distribution³⁰ and the excitation of overtone surface waves (E Okal, personal communication) seem to suggest that the faulting extended deeper than 100 km. Thus, the depth given here is probably the shallowest estimate.

unbending strain rate can easily be of the order of 10^{-17} to 10^{-16} s^{-1} (an unbending strain rate of 10^{-17} s^{-1} of a 100-km thick slab, subducting 10 cm yr^{-1} , corresponds to a change of the dip angle of $\sim 0.05^\circ/100 \text{ km}$, which is impossible to detect from seismicity⁸). On the other hand, the strain rate due to thermo-elasticity^{27,28} (10^{-18} to 10^{-17} s^{-1})²⁸ is too low to be the main cause of the double seismic zone. Although all the other proposed mechanisms may contribute to its occurrence, the double seismic zone can be satisfactorily explained by the simple

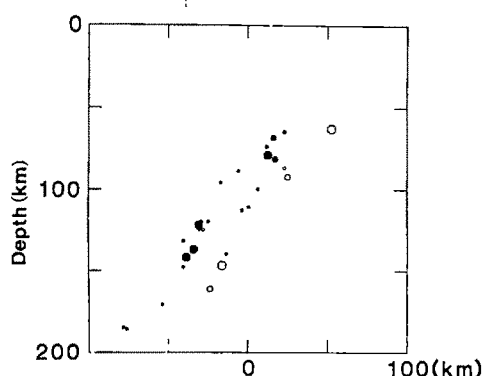


Fig. 4 To assign appropriate relative errors to the hypocentral parameters, a relative relocation was performed⁸. The method is similar to one used by Samowitz and Forsyth¹⁶, except that here only depth constrained events are used. Data are selected from the ISC bulletin from 1971 to 1979 with the following conditions; pP-P constrained depths between 60 and 200 km, >30 P arrival observations. For the first iteration, all events are relocated with the depths fixed at the pP-P depths using the P-wave arrival times reported at stations between 30° and 100° distance and ISC locations as the starting guesses; 440 stations were used. After the first iteration, the stations which have arrivals from >10 earthquakes are selected and assigned station adjustments as a linear function of distance between stations and earthquakes by the least squares method. This leaves 113 stations. For the second iteration, travel times are first adjusted by using the station adjustments estimated in the first iteration. Each event was then relocated separately using only the arrivals from those 113 stations. The estimated relative errors (90% confidence intervals) are usually smaller than the largest circles. Symbols are as in Fig. 3. The smallest circles denote events for which no information about their focal mechanism is known. Although a clear separation of two seismic zones seems to exist, it could be coincidence¹⁷. Therefore, what is most important is that the down-dip compressional events are still always located above the down-dip tensional type events even after the relocation.

model of geometrical unbending. The cause of this unbending is uncertain and probably varies from place to place. For example, pressure due to mantle flow²⁹ (or equivalently, dynamical sagging²⁵) or the weight of the slab itself can unbend the slab.

For a given magnitude of the strain rate, the distance between the two seismic zones depends strongly on the rheological properties and the thermal structure of the descending lithosphere²⁶. Thus, as pointed out by Tsukahara²⁶, studies of the fine structure of double seismic zones in many areas will give important new information on the thermal and rheological properties of the subducting lithosphere⁸.

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The influence of subduction processes on the geochemistry of Japanese alkaline basalts

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The composition of volcanic rocks erupted in complex plate tectonic settings can provide information on the nature of the underlying mantle. We show here that the geochemistry of alkali basalts from Japan and eastern Asia varies systematically with distance from the Japanese island-arc. Samples from northeastern Japan, relatively close to the Japan Trench, are enriched in K, Sr, Ba and Rb and depleted in Ta, Nb and Ti as compared with samples from southwestern Japan. Both sets show an island-arc influence on their composition, but alkali basalts from still further west (Korea and northeastern China) have chemistries which are indistinguishable from ocean island basalts. We suggest that the north-eastern island-arc type of alkali basalts were derived from a 'normal' upper mantle source altered by fluids or melts released from the underlying subducted Pacific plate. The extent of this island-arc-related alteration decreases with distance from the trench.

Major and selected trace-element concentrations of 200 Tertiary to Recent alkali basalts were determined by a combination of X-ray fluorescence (XRF) and instrumental neutron activation analysis (INAA) using the method described by Barnes and Gorton¹. Most of the samples analysed are nepheline normative and have the following characteristics: SiO₂ contents of less than 52 wt%, Ni contents of greater than 100 p.p.m., Cr contents greater than 150 p.p.m. and no detectable Eu anomalies. Samples with these characteristics are assumed to have retained their primitive chemistry. Olivine ± clinopyroxene fractionation may have produced a slight increase in the concentration of the incompatible elements in some samples but any change in ratios of these elements will be small.

Selected analyses are plotted on normalized abundance pattern (NAP) diagrams²⁻⁵ (Fig. 1). These diagrams give the concentration of selected incompatible elements normalized to their concentration in the primitive mantle. The elements are arranged systematically in order of increasing incompatibility, with the most incompatible elements plotted on the left. Figure 1a gives NAPs for an average depleted mid-ocean ridge basalt^{3,4}, typical Hawaiian tholeiite⁶ and alkali basalt⁶, an average continental alkali basalt from Kenya⁷, an average island-arc calc-alkaline basalt⁴ and an average arc tholeiite⁴. Both island-arc rock types are characteristically enriched in K, Ba, Sr and Rb and depleted in Ta, Nb and Ti (weakly) relative to adjacent elements in the NAP diagram.

NAP diagrams for samples from Rishiri Island (northeastern Japan), southwestern Japan, Jejudo Island (Korea) and Datong (northeastern China) are plotted in Fig. 1b, c and d respectively. They can be divided into three types: an island-arc type enriched in K, Ba, Sr and Rb and depleted in Ta or Nb and Ti, a transitional type which shows a much weaker island-arc signature, and a normal type with a NAP typical of ocean island and continental alkali basalts. The island-arc type is confined

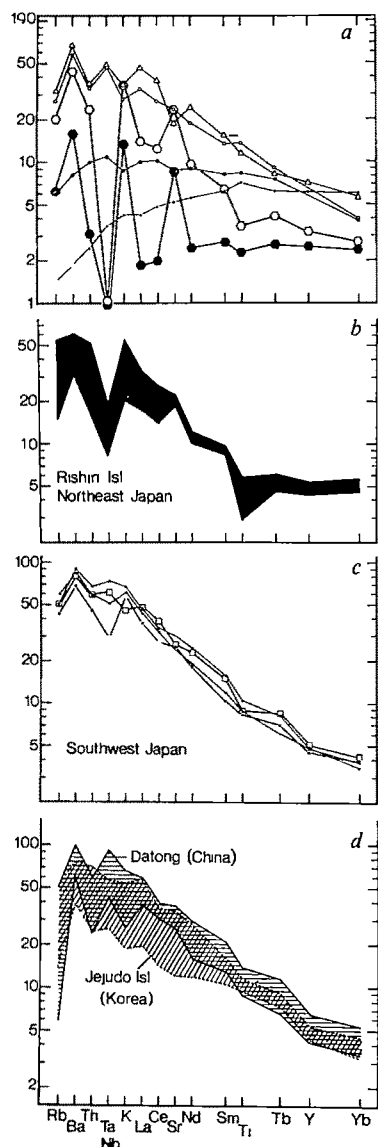


Fig. 1 *a*, Normalized abundance pattern (NAP) diagram for a typical ocean island alkali basalt⁶ (○) and tholeiite⁶ (●) from Hawaii, average continental alkali basalt (Δ) from Kenya⁷, average island-arc calc-alkali basalt⁴ (○) and tholeiite⁴ (●) and average depleted mid-ocean ridge basalt⁴ (+). *b*, NAP diagram showing the range of value for six alkali basalts from Rishiri Island, northeastern Japan. The patterns show a strong island-arc signature. *c*, Selected NAPs for transitional alkali basalts from southwestern Japan. The patterns were selected to show the highest K^* value [1.51 (×)], the lowest value [0.84 (□)] and two intermediate values (●). *d*, Variations in the NAPs for normal alkali basalts from Datong, northeastern China and Jejudo Island, Korea. Note all element abundances in *a*–*d* are normalized to their concentration in the primitive mantle.

to northeastern Japan, the transitional type to southwestern Japan and the normal type to Korea and northeastern China.

The geographical distribution of the three types of alkali basalt is summarized in Fig. 2 where the average value of $K^* = 2[K/(Ta + La)]_n$ (abundances of all three elements are normalized to primitive mantle values) is plotted for the alkali volcanic centres studied in this project and for primary magma compositions of sub-alkali volcanic centres studied by Sakuyama and Nesbitt⁸. This ratio was chosen because Ta and La are adjacent to K on the NAP diagram. K^* can be taken as a measure of the amount of K enrichment and therefore the intensity of the island-arc signature. As shown in Fig. 1*a*, ocean island and continental alkali basalts are typically depleted in K relative to La and Ta (that is, $K^* < 1$).

K^* values for primitive of sub-alkali basalts from northeastern Japan are greater than 2.8, with highest values occurring closest

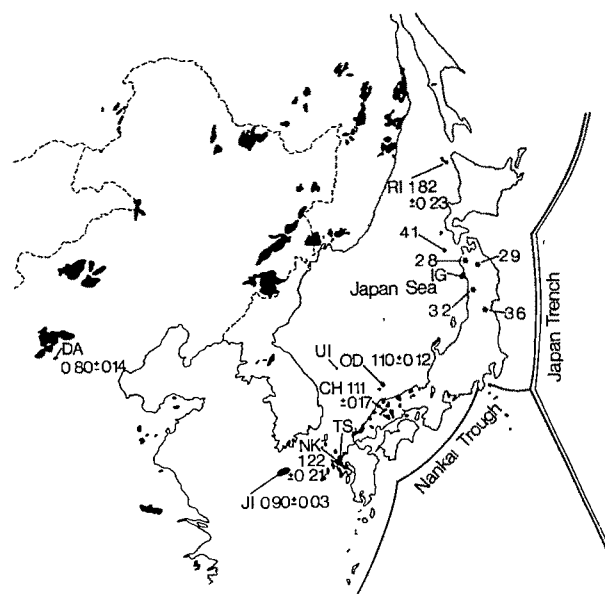


Fig. 2 A map of the Japan Sea area showing distribution of Cenozoic alkali basalts and $K^* = 2[K/(Ta + La)]_n$ values for each centre, with standard deviation for the alkali volcanic centres studied. Additional data from Sakuyama and Nesbitt⁸ for one alkali (●) and several tholeiitic and calc-alkaline volcanic centres (*) in northeastern Japan are also plotted. Note that northeastern Chinese Cenozoic basalts³⁶ have K^* values < 1 (negative K anomalies). Symbols: RI, Rishiri Island; IG, Ichinomegata; OD, Oki-Dogo; CH, Chugoku; NK, northwestern Kyushu; TS, Takashima; JI, Jejudo Island; UI, Ulungdo Island; DA, Datong

to the Japan Trench. The highest K^* value for alkali basalts from northeastern Japan (4.1) is also from the centre closest to the trench, with the value for Rishiri Island, further from the trench in this area, being appreciably less. The average value of K^* for centres from southwestern Japan varies between 1.1 and 1.2, although individual samples may have values as low as 0.84. Samples from Korea and northeastern China invariably have values below 1.0. Figure 2 shows that K^* values for alkali basalts decrease away from the Pacific plate trench. Variations in Ba, Sr and Rb abundances show similar trends to the K^* values. The concentrations of major elements and all other trace elements, including Zr, Y and rare earth elements (REE), are similar for all types of alkali basalts in the area studied.

The observation of decreasing K^* values away from the Pacific plate trench is apparently at odds with Kuno's study⁹ which showed that that K_2O content of Japanese volcanic rocks increase with distance from the trench. We interpret the increase in K_2O to be due to decreasing degrees of partial melting and the change in K^* values to be due to a decrease in subduction-related alteration of the mantle with increasing distance from the trench. Thus the two observations are not incompatible.

The lack of a significant difference in major and trace elements (Th, Zr, Y and REE) for samples from Japan, Korea and northeastern China suggests that the alkali basalts from each of these areas formed by similar processes. If this is correct, the observed enrichments in Ba, K, Sr and Rb must reflect differences in the concentration of these elements in the source. Since the Japanese alkali basalts are found above the subducted Pacific plate except in the northwestern Kyushu area, the obvious explanation for the differences is that the mantle above the subduction zone has been contaminated by K, Ba, Sr and Rb bearing metasomatic solutions and/or partial melts derived from the slab^{10,11}.

Alkali basalts from southwestern Japan show little evidence of an island-arc signature in spite of their proximity to the Philippine Sea plate subduction zone. This is a young subduction zone and the angle of subduction is shallow^{12–14}. We believe that the island-arc type geochemical signature is absent from

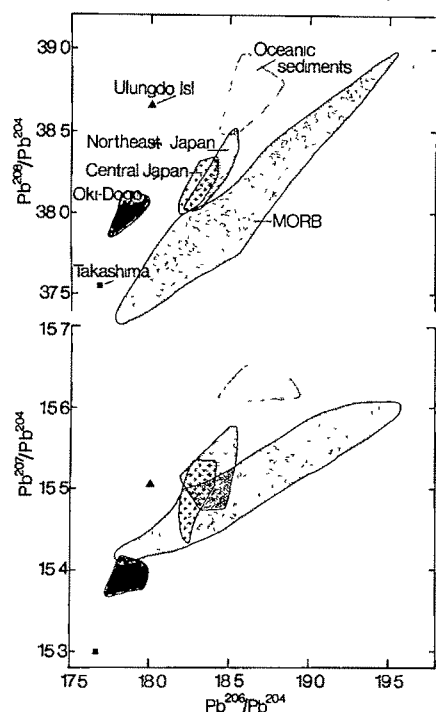


Fig. 3 Lead isotope compositions of Japanese alkaline, tholeiitic and calc-alkaline basalts¹⁸⁻²⁰, oceanic sediments^{3,37,38} and MORB^{39,40}. All those analyses carried out by the PbS method were recalculated to 'absolute values' for comparison with those analysed by the silica-gel method using a correction factor of -0.35% per mass unit. The data for Ulungdo Island are unpublished analyses by D. Nelson (personal communication). Pb isotopic compositions of basalts in northeastern and central Japan show a mixing relationship between oceanic sediments and MORB, and become less radiogenic with increasing distance from the Japan Trench. In central Japan, the compositional variation with distance is less regular because of the small difference in the distance between volcanic centres. However, the volcanic centre nearest to the trench, Ohshima Island, has the most radiogenic Pb composition. Pb isotope compositions of alkali basalts from southwestern Japan are similar to basalts from Hawaii. In spite of having similar $^{206}\text{Pb}/^{204}\text{Pb}$ ratios to MORB, their $^{208}\text{Pb}/^{204}\text{Pb}$ ratios are much higher than MORB, suggesting an evolutionary history with a higher Th/U ratio and a more primitive source.

this area because the contaminated mantle above the Philippine Sea plate has not reached sufficient depth to be involved in the production of alkali basalts.

Our conclusion that the alkali basalts of northeastern and southeastern Japan have come from mantle sources which have undergone different degrees of subduction-related alteration is supported by studies of mantle xenoliths. Xenoliths from Ichinomegata, northeastern Japan, have abundant hydrous minerals¹⁵ and contain water-rich fluid inclusions^{16,17}, whereas those from southwestern Japan contain a high-temperature, nearly anhydrous mineral assemblage¹⁵. These observations are consistent with the hypothesis that the mantle source of Japanese alkali basalts is strongly contaminated in the north-east, but only weakly contaminated in the south-east.

Further support for this interpretation is provided by available Pb¹⁸⁻²⁰, Sr^{21,22} and Nd²² isotopic studies. Basalts from northeastern and central Japan have a strong component of Pb from oceanic sediments (Fig. 3) which decreases with distance from the trench, that is, the sedimentary Pb component is the strongest in arc tholeiites and weakest in alkali basalts. In contrast, alkali basalts from southwestern Japan show Pb systematics similar to basalts from nonradiogenic ocean islands such as Hawaiian tholeiites²³. These observations suggest that the fluids (or melts) which contaminated the mantle in northeastern Japan had a strong sedimentary component and that this component decreased in importance with increasing distance from the Pacific plate trench. The implication is that the sedimentary components which contaminate the overlying wedge are con-

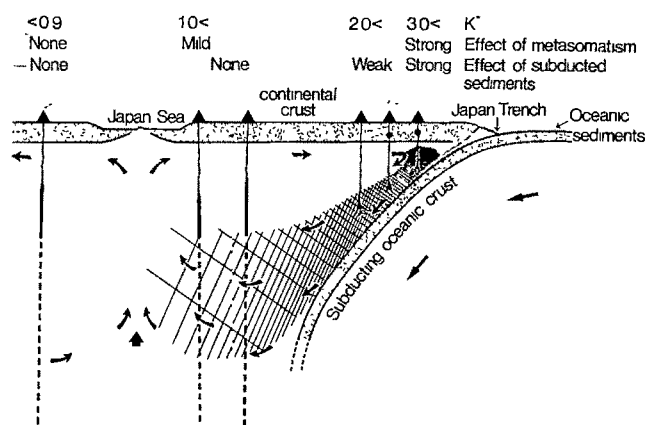


Fig. 4 A diagrammatic model of magma generation above the Japanese subduction zone. The subducted slab dehydrates or melts as it descends, releasing a fluid which contaminates the overlying mantle (cross-hatched zone). The intensity of this contamination decreases with increasing distance from the trench. Oceanic sediments (dotted zone) are also carried down by the subducted slab, but dehydrate (or melt) at shallow depths so that their influence is confined to volcanic centres close to the trench. All volcanic rock types from northeastern Japan have come from a strongly contaminated MORB-type source. The alkali basalts of southwestern Japan are believed to originate deeper in the mantle from an OIB-type source and to have interacted chemically with an overlying contaminated MORB-type mantle during their ascent. Alkali basalts from China and Korea were probably produced by a similar process but did not interact with a subduction-contaminated mantle.

sumed at relatively shallow depths. Sr and Nd isotopic studies of basalts from northeastern Japan also require the mantle to be contaminated by a component derived from subducted oceanic sediments^{4,22}.

Figure 4 is a summary diagram which relates our geochemical data and the available isotopic data for Japanese volcanics to existing petrological²⁴⁻²⁹ and convection models³⁰⁻³² for island-arc systems. Pb, Sr and Nd isotopic studies require at least two types of mantle to produce the volcanic rocks of Japan; a mid-ocean ridge (MORB) type and an ocean island (OIB) type. The tholeiitic and alkalic volcanics of northeastern Japan required a MORB-type source which has been strongly contaminated by fluids and/or melts derived from the subducted Pacific plate. The intensity of this contamination decreases with distance from the trench. We suggest that the plumes that give rise to the alkali volcanics of southwestern Japan originate from an OIB-type source at a much greater depth. As the ascending plumes rise, they assimilate some of the overlying contaminated mantle and, in doing so, acquire a weak island-arc geochemical signature. The alkali basalts of northeastern China and Korea show no evidence of an island-arc signature and are geochemically indistinguishable from ocean island alkali basalts. Chemical and isotopic variations observed in these alkali basalts may have resulted from interaction between heterogeneous plume source material and the continental lithosphere.

The absence of a sedimentary Pb component from alkali basalts of southwestern Japan, which show a mild island-arc geochemical signature, is difficult to reconcile with a geochemical model which requires oceanic sediments to be subducted in an island-arc system and recycled through the mantle^{33,34}. Our observations suggest that the sedimentary component is consumed at shallow depths during subduction through repeated dehydration and partial melting as the oceanic lithosphere sinks into the mantle. Sedimentary components may be recycled into the mantle as a consequence of subduction related to continental collision³⁵, recycling of the mantle wedge above the subduction zone or delamination and recycling of the lithospheric mantle³⁵ previously processed through the island-arc environment.

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Chugoku, Rishiri Island, Jejudo Island, Ulungdo Island and Datong respectively. S-s.S. publishes with permission of the Director of Bureau of Mineral Resources Australia. We also thank A. W. Hofmann for his critical comments.

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Microzones surrounding phytoplankton form the basis for a stratified marine microbial ecosystem

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Although there are 10^7 – 10^9 bacteria per litre of sea water and up to 50% of marine primary productivity passes through them¹, no direct information exists on small-scale spatial distributions of individual bacteria. It has been proposed that bacteria cluster around particulate nutrient sources²⁻⁴, but evidence for this is indirect, based on the presence of chemotaxis in isolates and a broad range— 10^{-9} – 10^{-4} M—of uptake affinity constants⁵. These data have led to the suggestion that there exist oceanic microzones having utilizable dissolved organic carbon (uDOC) orders of magnitude above background concentrations⁶. Here, our calculations of size (10^{-1} cm), utilizable DOC concentrations (10^{-9} – 10^{-7} M) and lifetimes of these nutrient gradients suggest that bacteria cluster around phytoplankton in low-turbulence layers and sheets comprising the thermocline. There, phytoplankton may sink slowly enough for bacteria to keep up with them, and mixing times are long enough to allow nutrient gradients to develop and bacteria to track these gradients. However, very favourable conditions are required for microzone concentrations to be significantly above background.

The size of the nutrient field was calculated from the excretion rate per cell and the dispersion of nutrients away from the cell.

We assumed that 40% of a phytoplankter's primary production is excreted; this represents a generous intermediate value from the wide range cited in the literature^{5,6}. We used high values for phytoplankton productivity⁷ (10^{-4} g C per l per day, over a 12-light period) and cell concentration⁸ (10^6 cells per l) (a more typical value is 10^{-5} g C per l per day). Based on recalculations of Chrost and Faust's⁸ data, we used 100 g as the weight of carbon per mole, and this indicates that 85% of the molecules that phytoplankton excrete have a relative molecular mass <500; amino acids and sugars are the major components of this size fraction⁹. These values result in a total flux out of a cell of 10^{-17} mol s⁻¹.

From the total nutrient flux per cell and the diffusivity of nutrient away from the cell, we determined the dimension of the microzone around a phytoplankter using equation (1)¹⁰

$$\text{Radius} = \frac{(\text{total flux})}{(4\pi)(\text{concentration})(\text{diffusivity})} \quad (1)$$

Here, radius means the distance at which the nutrient gradient emanating from the cell is enhanced 10% above the background concentration. We used a background concentration of 10^{-8} M or 10^{-11} mol cm⁻³ (ref. 11), a relatively low value that again favours microzones (a more typical value, including carbohydrates, is 10^{-7} M; ref. 12). Consequently, 10% of the background level is 10^{-9} M (represented by concentration in equation (1)). Equation (1), using 10^{-5} cm² s⁻¹ for diffusivity¹³, gives a radius of 8×10^{-2} cm or ~1 mm. At this distance from the cell the concentration is theoretically 1.1×10^{-8} M, compared with 1×10^{-8} M for background. For the more typical values cited above, the radius is < 10^{-3} cm.

To determine the significance of the larger microzones, we are interested in the complete range of concentrations. The concentration at the phytoplankter surface represents the maximum enhanced concentration. To estimate this maximum surface concentration, equation (1) was rearranged, and we assumed a relatively small diatom radius of 5×10^{-4} cm to minimize surface area and thus maximize surface concentration (a more typical value is about three times as large). The resulting maximum concentration, under optimum conditions, is $\sim 10^{-7}$ M. For the more typical values cited above, this maximum is $\sim 5 \times 10^{-9}$ M, or barely above background. Thus, if much higher concentrations exist in the literature, diffusion must be restricted by a physical barrier, such as mucus.

One must also compare the formation time of the zone with its rate of disintegration by turbulence. Based on Carslaw and Jaeger¹⁰, we calculate that it takes at least 10^2 s for the leading edge of the excreted material to reach a radius of 1 mm. Table 1 demonstrates that nutrients are dispersed long before reaching a radius of 1 mm, except in low-turbulence conditions such as

Table 1 Lifetimes of Kolmogorov eddies within the range of turbulence found in the ocean

Energy (E) dissipation (cm ² s ⁻³)	Eddy			
	Length (cm)	Velocity (cm s ⁻¹)	Lifetime (s)	Typical location
10^{-2}	0.10	0.10	1.0	Mixed layer
10^{-3}	0.18	0.056	3.2	Mixed layer
10^{-4}	0.32	0.030	10	
10^{-5}	0.56	0.018	32	
10^{-6}	1.0	0.010	100	Thermocline
10^{-7}	3.2	0.0056	320	Thermocline

Kolmogorov eddies are the hydrodynamic 'units' which contain a phytoplankter and its microzone. The necessary equations are from Batchelor²⁷. Values are from Ozmidov²⁸ and from Gregg²⁹. Length = $(v^3/E)^{1/4}$; velocity = $(vE)^{1/4}$; lifetime = $(v/E)^{1/2}$, where v = kinematic viscosity of water (10^{-2} cm² s⁻¹)

the thermocline. The thermocline is composed of 1–5-m-thick 'layers', regions of minimal turbulence separated by 1–10-cm-thick sheets of near-laminar flow¹⁴. Sheets and layers also characterize the 'mixed' layer on calm days¹⁴. We suggest that sheets and layers are the most likely location for microzones for two reasons: (1) in these areas a nutrient gradient has time to reach steady state around a phytoplankter, and (2) bacteria have time to track this gradient chemotactically. The latter point is explained below, but essentially bacteria cannot move by chemotaxis fast enough to form a cluster in less than ~100 s.

For bacteria to cluster around a phytoplankter, they must find, enter and stay within the nutrient gradient. Bacterial sensitivity to chemical gradients^{15,16} and their ability to orient to them is well documented^{17,18}. However, in conventional chemotaxis studies, the nutrient source is stationary and the bacteria are given minutes to hours to orient^{2,19}. Motile bacteria swim, stop, randomly reorient, and swim again. Bacteria approach a nutrient source because runs between stops are longer when they move up a gradient than when they move down one¹⁷. Random reorientation means that bacteria move discontinuously up a gradient in a biased random walk. Consequently, net movement in a gradient is only ~1–3 $\mu\text{m s}^{-1}$, about an order of magnitude slower than their average swimming speed of 10–30 $\mu\text{m s}^{-1}$ (ref. 20). These values derive from laboratory studies of enteric bacteria. The rates for marine bacteria are probably similar, but unknown; if they differ significantly our conclusions are altered accordingly.

It is important to compare the rate of bacterial chemotaxis with the motion of phytoplankton. In the ocean's mixed layer an average non-motile phytoplankter, such as a diatom, sinks at a rate of ~10 $\mu\text{m s}^{-1}$ (1 m per day; ref. 21) or about three times the speed at which a bacterium can move chemotactically towards a nutrient source. This suggests that bacteria have difficulty in keeping up with most diatoms and probably cannot overtake or maintain themselves within the nutrient gradient, making clustering of bacteria to form microzones unlikely in the mixed layer. We chose sinking as an example of cell movement because the average values are well documented²¹. But any movement of the nutrient source (relative to the immediately surrounding water) that is more rapid than the bacterial chemotactic rate (~3 $\mu\text{m s}^{-1}$) prevents clustering. Swimming motility is particularly rapid at tens to hundreds of micrometres per s (ref. 22). This supports our hypothesis that microzones most probably occur in the thermocline, as phytoplankton sinking rates are reduced in this region²³. Also, Woods^{14,24} reports that sheets remain turbid while the surrounding water is clear, suggesting that particles are concentrated in the sheets.

The long eddy lifetimes in reduced turbulence may be important to other small-scale processes, such as formation of micro-scale nutrient patches²⁵. Longer eddy lifetimes may allow chemotactic swarming of motile phytoplankton around a zooplankton or nutrient particle. This potential behavioural response may explain how Lehman and Scavia's²⁵ results were possible despite Jackson's²⁶ calculations predicting otherwise.

In summary, generous estimates biased toward high nutrient concentrations show that: (1) significant organic enrichment extends less than 1 mm from a phytoplankter, and (2) the highest concentration, at the cell surface, is enhanced less than 10 times above background. Estimates from the more typical conditions yield apparently negligible microzones. Furthermore, bacteria cannot keep up with phytoplankton moving faster than the bacterial chemotactic swimming rate. The swimming speeds of motile phytoplankton, and average sinking rates of non-motile forms within the mixed layer, appear to preclude clustering. We suggest that clustering is limited to non-motile forms such as diatoms and seems most likely, and perhaps best studied, in the thermocline. There, hydrodynamic fine structure contributes to reduced phytoplankton sinking rates and provides an environment more amenable to maintenance of ordered, small-scale interactions between microbes than is present in the turbulent mixed layer. This may lead to a stratified microbial community across and within the thermocline.

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Female mimicry in garter snakes

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In many diverse taxa, males of the same species often exhibit multiple mating strategies¹. One well-documented alternative male reproductive pattern is 'female mimicry', whereby males assume a female-like morphology^{2–8} or mimic female behaviour patterns^{9,10}. In some species males mimic both female morphology and behaviour^{2,7,11,12}. We report here female mimicry in a reptile, the red-sided garter snake (*Thamnophis sirtalis parietalis*). This form of mimicry is unique in that it is expressed as a physiological feminization. Courting male red-sided garter snakes detect a female-specific pheromone and normally avoid courting other males. However, a small proportion of males release a pheromone that attracts other males, as though they were females. In the field, mating aggregations of 5–17 males were observed formed around these individual attractive males, which we have termed 'she-males'. In competitive mating trails, she-males mated with females significantly more often than did normal males, demonstrating not only reproductive competence but also a possible selective advantage to males with this female-like pheromone.

On emergence from the winter den, female red-sided garter snakes are usually courted by 10–20, but sometimes as many as 100 males simultaneously, although only one male mates with the female¹³. Males begin to court females when rapid tongue-flicks by the male deliver pheromone cues from the female's dorsal surface to the male's vomeronasal organ^{14,15}. During the spring of 1983, a census was taken of presumed 'mating balls' at a study site in central Manitoba, Canada. In 33 of the observed 200 (14%) mating balls studied, no female was found; instead a single male was being courted by the other males. These

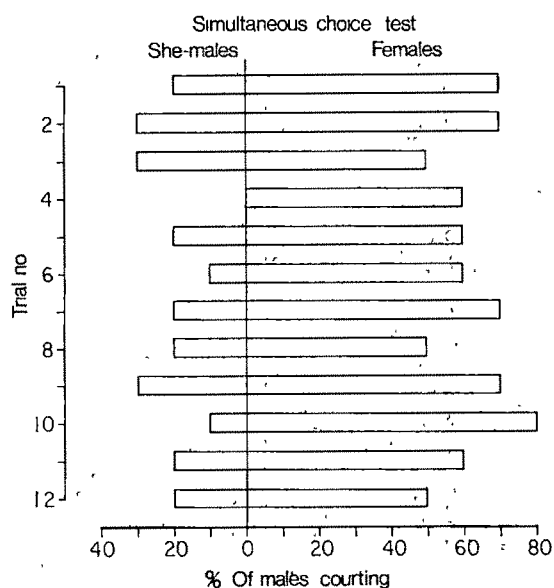


Fig. 1 Results of a simultaneous choice test. In this test a female and a she-male were placed in an arena with 10 courting males. After 5 min the number of males that courted either stimulus animal was recorded.

courted males (she-males) possessed normal hemipenes, testes and accessory sex structures and their mean snout-vent length and body weight did not differ significantly from those of other males taken at random from the population.

The attractiveness of she-males is not caused by prior physical contact with recently emerged females. Males rubbed against a recently emerged, attractive female were not courted by other males, agreeing with field and laboratory observations in which recently mated males that have been in contact with females for up to 1 h have never been observed to be courted. The attractiveness of the she-males appears to be as permanent as that of sexually mature females¹⁶. All of the she-males tested ($n=33$) elicited courtship from sexually active males when tested at 3, 18 and 40 weeks after capture.

Steroid hormone levels were assayed on blood samples taken in the field from males, females and she-males¹⁷. Analysis of variance revealed that females had significantly higher circulating levels of oestradiol than either males or she-males ($P<0.05$, Student-Newman Keuls) (Table 1). Although testosterone levels in males and females were not significantly different, she-males had significantly higher levels of testosterone than either females or males ($P<0.05$, Student-Newman Keuls). There were no significant differences in circulating levels of dihydrotestosterone. Presumably, the high levels of testosterone in the she-males was a result of either higher secretion or reduced clearance from the circulation.

Table 1 Circulating levels of sex steroid hormone in the two male morphs and the female red-sided garter snake

	(n)	Hormone (ng ml ⁻¹)		
		Testosterone	Oestradiol	Dihydro-testosterone
Male	9	5.720 ± 1.655	0.061 ± 0.012	0.231 ± 0.066
Female	9	0.233 ± 0.082	7.703 ± 3.440	0.093 ± 0.017
She-male	9	19.785 ± 5.703	0.075 ± 0.013	0.142 ± 0.025
F ratio (d.f. 2, 24)		7.68	3.69	2.45
F ≥ 3.40, P > 0.05				

Plasma levels of dihydrotestosterone, oestradiol and testosterone were determined by steroid radioimmunoassay. Values are the mean ± s.e.m.

Table 2 Transfer of the attractiveness pheromone

Trial no.	Female	Male	She-male
1	+	—	+
2	+	—	+
3	+	—	+
4	+	—	+
5	+	—	+

Ten sexually active male red-sided garter snakes were placed in an arena at the den site. Pheromone samples obtained from the skin surface of females, males and she-males were then presented to the test males. A sample was scored as attractive if five or more males courted for 10 s. Ten different males were used in each trial.

The pheromone produced by the she-males is apparently indistinguishable from that produced by females. Samples obtained from the skin surface using the non-polar solvent toluene revealed that the skin of females and she-males, but not of males, elicited courtship behaviour from conspecific males at the den site (Table 2). However, in simultaneous choice tests ($n=12$) in which 10 courting males were able to choose between a female and a she-male, the majority of males preferred the female in every case, although in each trial some males also courted the she-male (Fig. 1). Thus, females may produce more of the pheromone or they may produce a qualitatively different pheromone from that of she-males.

Competitive mating trials were conducted in the field in which groups of five males and five she-males were selected at random and released into an arena (1 m × 1 m) with an unmated, attractive female. In a total of 42 mating trials, 29 resulted in a she-male mating with the female and 13 with a male mating; thus, she-males mated significantly more often than did normal males ($\chi^2=11.85$, $P<0.001$). In field observations, she-males joining an established mating ball caused courting males to ignore the female and begin to court the she-male. A possible advantage to the she-male's attractiveness thus lies in an ability to gain a better position in the mating ball by confusing other males.

Currently, breeding experiments and future field work are being designed to resolve an enigma: why are all males not she-males if she-males do indeed have a reproductive advantage over normal males?

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Related spotted hyaenas forage together but do not cooperate in rearing young

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Spotted hyaenas (*Crocuta crocuta*) cooperate little in raising their young¹, in contrast to other social carnivores²⁻¹¹. It has been contended that the average relatedness between adult spotted hyaenas in a clan is so low that there is no kin selection pressure in favour of helping to rear young¹². In the southern Kalahari, however, I found that there is a high degree of relatedness between the members of clans and that kin selection may be implicated in ways other than cooperation in raising young.

Two clans were intensively studied in the Kalahari Gemsbok (South Africa) and adjacent Gemsbok (Botswana) National Parks from January 1979 to April 1984. The animals were individually marked or were recognizable. The degrees of relatedness (r) between the members of the clans were calculated making the following assumptions: (1) Females suckled only their own cubs¹. (2) The degree of relatedness between the original three key adult females of the Kousaunt clan (Fig. 1), were equivalent to the mean of the known degrees of relatedness between 10 female pairs from the Kaspersdraai clan, that is 0.38. (3) The resident adult male in a clan at the time when cubs

were conceived was their father. When there were two adult males present (Kousaunt clan, May 1981 to August 1983; Fig. 1), the behaviour of all but one of the females (Guy) towards the second male (Necklace) suggested that they would not have allowed him to mate with them. Accordingly, the male Hans was assumed to have fathered all cubs born during this period, except for Guy's cub, which was not included.

Spotted hyaenas of the southern Kalahari live in clans of 5–20 individuals inhabiting territories of 600–1,900 km² (M.G.L.M., manuscript in preparation). All males ($n=13$) left their natal territories at 2½ to 3½ years old, but only 1 of 8 females did so. The average degree of relatedness between 231 pairs of hyaenas of the Kousaunt clan, which averaged 17 individuals during the study, was 0.29 and between 32 pairs of the Kaspersdraai clan, which averaged eight individuals, was 0.33.

The hyaenas' food consists primarily of large and medium-sized ungulates, 55.3% ($n=197$) of which they killed. Observations of spotted hyaenas hunting gemsbok (*Oryx gazella*) calves (43% of kills), sub-adults and adults (10% of kills) and blue wildebeest (*Connochaetes taurinus*) of all ages (15% of kills), revealed no correlation between hunting success and hunting group size (Fig. 2). Single hyaenas, however, were unable to kill large gemsbok, two and four being the smallest groups observed to catch sub-adults and adults, respectively. It was usually impossible to differentiate between hunting attempts on wildebeest calves and adults, but group size did not appear to be a large factor in determining hunting success, as Kruuk¹ also concluded. Furthermore, carrion, which is usually located by scent, can be located as efficiently by a single hyaena as by

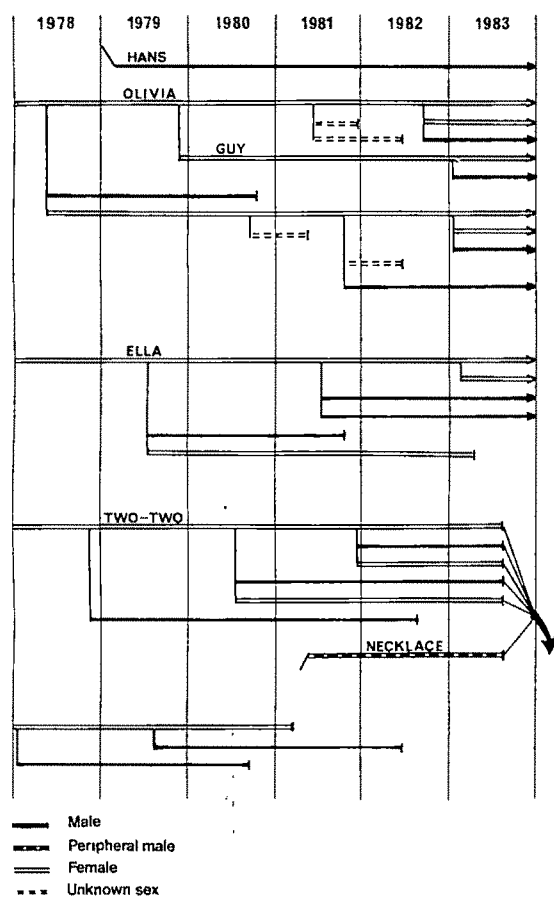


Fig. 1 Summary of the reproductive history of the Kousaunt clan from 1978 to 1983, showing the three coalitions and the subsequent fission of the clan. The birth of a litter is represented by a vertical line descending from the female's line, immigration of an individual is indicated by an oblique line. The ending of a horizontal line by one short vertical line denotes either death or emigration. The names of animals mentioned in the text are given.

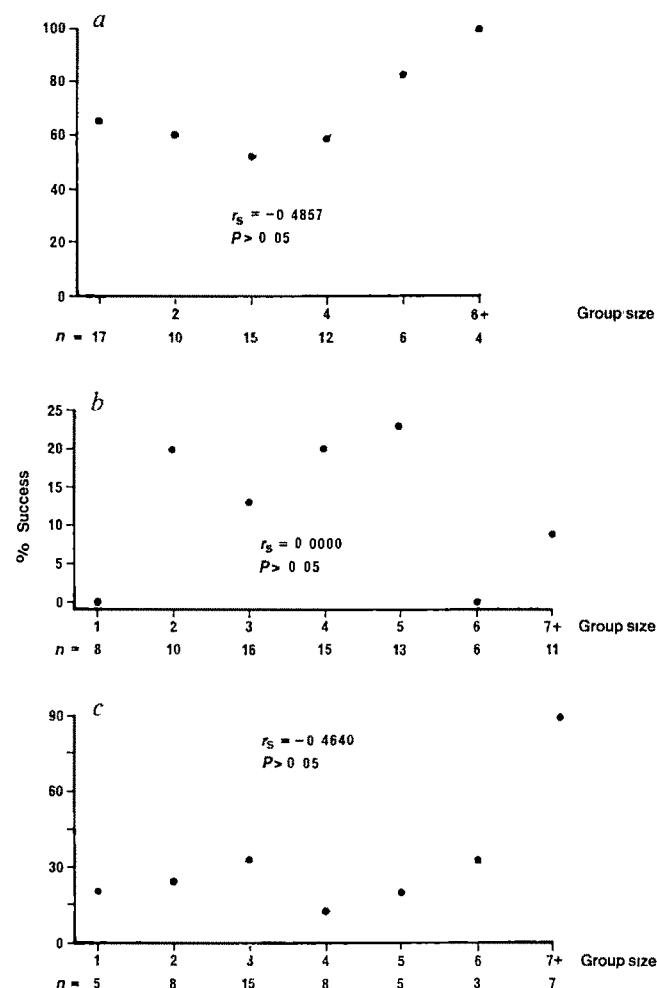


Fig. 2 The relationship between spotted hyaena hunting group size and hunting success of a, gemsbok calves, b, gemsbok sub-adults and adults; and c, wildebeest of all ages.

several. Of 566 foraging groups, 29.9% comprised one, 19.3% two, 18.7% three, 13.3% four, and 19.2% five or more animals. Foraging groups sizes, therefore, were often larger than needed for maximum foraging efficiency, so cooperation in hunting may not be the only selective pressure for group foraging in these spotted hyaenas.

Another reason for group foraging could be that larger groups are more successful at defending their food from competitors^{13,14}. In the southern Kalahari, however, spotted hyaenas and their chief competitors, lions (*Panthera leo*), are few. Only once did I observe other hyaenas (and on three occasions, lions) trying to appropriate a carcass from feeding spotted hyaenas ($n = 197$).

Could spotted hyaenas form these larger foraging groups to allow closely related individuals access to food, thus possibly enhancing their inclusive fitness? The degrees of relatedness between the members of 131 foraging groups of Kousaunt hyaenas and 28 foraging groups of Kaspersdraai animals, were compared with those for equal numbers of equal-sized foraging groups randomly generated by a computer from the pool of available hyaenas in each clan. In both cases the degrees of relatedness in the foraging groups were significantly higher than they were in the random groups (Kolmogorov-Smirnov one-sample test: Kousaunt, $D = 0.227$, $P < 0.01$, two-tailed test; Kaspersdraai, $D = 0.149$, $P < 0.01$, two-tailed test). These hyaenas, therefore, showed a strong tendency to preferentially forage with their close kin.

Certain individuals were often excluded from food. I compared the degrees of relatedness of only those members of 32 feeding groups from the Kousaunt clan that were permitted to eat at a given carcass, with those of 32 random groups from the clan, chosen as described above. Here again the degrees of relatedness were significantly higher in the observed groups than in the random groups (Kolmogorov-Smirnov one-sample test, $D = 0.088$; $P < 0.01$, two-tailed test).

In 1983 a split occurred in the expanding Kousaunt clan (Fig. 1). Three coalitions comprising the three surviving original adult females plus their offspring (Fig. 1) developed in the clan (unpublished observations); this reached a climax in August 1983 when all the members of the Olivia and Two-Two coalitions were observed to engage in a fierce intra-clan fight. The battle probably was not their first as, prior to this, two members of the Olivia coalition had sustained injuries. After the battle the Two-Two coalition, together with the male (Necklace) (Fig. 1) departed and settled in an adjoining area. The Olivia and Ella coalitions continued to inhabit the same area and den communally.

Relatedness between individuals, therefore, is more important in spotted hyaena society than was thought previously. Apart from sharing dens, why then do they not cooperate in raising young as brown hyaenas (*Hyaena brunnea*) do^{10,11}? Regurgitation of food for young is not seen in the Hyaenidae, probably because of the rapid rate at which food passes from the stomach to the intestines¹. Food carrying, which is observed in the brown hyaena, would be difficult to accomplish in spotted hyaenas¹⁵ as: (1) much of their food consists of large carcasses, which the hyaenas often have to travel long distances to obtain; (2) the social nature of their feeding habits¹ means that there is often nothing suitable left to take back to a den; and (3) at the communal dens the larger cubs would get most of the food. Cubs, therefore, rely heavily on their mother's milk during their first year¹; this imparts a heavy nutritional load on the females, which may only be able to afford to suckle their own offspring. Finally, the small den tunnels^{1,16,17} provide ideal protection for cubs when the adults are away, thus precluding the need to leave guards. I suggest that spotted hyaenas cooperate little in raising their young because of ecological pressures and not because of low levels of relatedness between clan members.

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Defective ability to self-renew *in vitro* of highly purified primitive haematopoietic cells

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Stromal cells play a critical role in haematopoiesis, both in a permissive and, probably, in a directive manner¹⁻⁵. Study of the interactions between stromal cells and haematopoietic stem cells, however, is difficult to perform using whole bone marrow, in which stem cells are indistinguishable from precursor cells and maturing haematopoietic cells, and where stromal and haematopoietic cells co-exist in a heterogeneous mixture. We have purified primitive haematopoietic spleen colony-forming cells (CFU-S) using fluorescence-activated cell sorting (FACS) and produced CFU-S populations which approach 100% purity (ref. 6 and B.I.L. and E.S., in preparation). This cell population is devoid of significant stromal cells and mature haematopoietic cells. Here, we report that when purified CFU-S are seeded onto a stromal adherent layer *in vitro*, foci of haematopoietic cells develop within the stroma followed by production of a wave of maturing and mature progeny. However, self-renewal of CFU-S does not occur and haematopoietic activity rapidly declines, indicating that caution should be applied in the use of highly purified stem cells for human bone marrow transplantation.

Irradiated long-term marrow culture adherent layers were used as the source of inductive haematopoietic stroma². We seeded the stroma (or empty culture flasks) with various numbers of normal marrow cells (Table 1). Normal bone marrow cells (5×10^5 containing 130 CFU-S) plated into an empty tissue-culture flask produced two small stromal cell foci per flask and no haematopoietic cells over 7 weeks. The same number of normal bone marrow cells plated onto an irradiated adherent layer led to the production and release of CFU-S into the growth medium for several weeks. Note that this is an underestimate of the total CFU-S as this does not include the CFU-S lodged in the adherent cell layer⁷. It is not known why haematopoiesis was exhausted in these cultures after 7 weeks. A larger inoculum of normal bone marrow cells, 5×10^6 cells, used to initiate a long-term bone marrow culture adherent layer, produced a confluent stromal adherent layer that promoted the maintenance of relatively few CFU-S for a limited duration, as described previously⁸. However, when 5×10^6 normal bone marrow cells were seeded onto an irradiated adherent layer, many CFU-S were produced for several weeks and the duration of haematopoiesis was clearly extended. In agreement with previous studies, fully competent long-term bone marrow cultures could be established from 1.5×10^7 normal bone marrow cells

Table 1 CFU-S production in irradiated marrow stromal cell layers and control flasks inoculated with normal marrow cells

Input normal bone marrow	Input CFU-S	Irradiated stromal cells	CFU-S per culture (release to growth medium) (weeks)						
			1	2	3	4	5	6	7
5×10^5	130	No	0	0	0	0	0	0	0
5×10^6	1,300	No	91	20	30	20	10	0	13
1.5×10^7	13,000	No	700	386	432	576	580	624	394
0	0	Yes	0	0	0	0	0	0	0
5×10^5	130	Yes	18	39	101	40	92	16	0
5×10^6	1,300	Yes	139	250	650	250	130	ND	100

To establish irradiated marrow stromal cell layers, we set up long-term bone marrow cultures as described previously¹. Briefly, each culture was initiated by flushing the contents of one femur (1.5×10^7 cells) from (C57BL/6 $\times$ DBA/2) F₁ donors into 10 ml Fischer's medium (Gibco) supplemented with horse serum (20% v/v) (Flow Labs) and 10^{-6} M hydrocortisone sodium succinate. Cultures were fed weekly by removing 5 ml growth medium and adding 5 ml fresh medium. After 4 weeks the cultures were irradiated with 1,500 rad ¹³⁷Cs, followed by a total change of medium 6 h later. The dose kills all haematopoietic cells yet spares relatively quiescent stromal cells and provides a nude inductive stroma that can function to promote the growth of haematopoietic cells for at least several weeks². The stromal cell layers were gently rinsed with Fischer's medium 7–10 days after irradiation and the appropriate number of fresh bone marrow cells was added in 10 ml Fischer's medium with 20% v/v horse serum. At weekly intervals, cultures were fed and the suspension cells collected during feeding were diluted (if necessary) and assayed for CFU-S according to the method of Till and McCulloch¹². Recipient mice were killed after 10 days. In some experiments, fresh bone marrow cells were seeded into empty culture flasks. ND, not done.

Table 2 CFU-S production in irradiated marrow stromal cell layers inoculated with FACS-sorted bone marrow cells

Input FACS-BM	Input CFU-S	Irradiated stromal cells	CFU-S per total culture (weeks)		
			1	2	3
10,000	700	Yes	—	19	—
5,000	350	Yes	—	—	0
2,500	175	Yes	22.5	8.5	—
1,250	87	Yes	—	—	0
625	44	Yes	9	2	4
312	22	Yes	—	—	4
20	1.3	Yes	—	—	0
500	26	No	0	0	—

Irradiated long-term bone marrow cultures were established as described in Table 1 legend. Normal bone marrow cells were enriched for CFU-S content by a modification (B.I.L. and E.S., in preparation) of the electronic sorting technique described by Visser *et al.*⁶ Normal bone marrow cells were labelled with $2 \mu\text{g ml}^{-1}$ fluorescein isothiocyanate-conjugated wheat-germ agglutinin (WGA-FITC) and subjected to a density cut in metrizamide. Cells with density $\leq 1.080 \text{ g ml}^{-1}$ were collected, washed in phosphate-buffered saline and 'sorted' on a fluorescence-activated cell sorter (FACS IV; Beckton & Dickinson). Electronic windows were set to select highly WGA-FITC-labelled cells with moderate forward and low perpendicular light scatter. The selected cells were collected in Fischer's medium containing 10% horse serum, washed and re-sorted under the same criteria as before. WGA-FITC was removed from the re-sorted cells by incubating for 15 min at 37 °C in isotonic, 0.2 M *N*-acetyl D-glucosamine⁶ before assaying the processed cells for CFU-S content¹². Recipient mice in the CFU-S assay were killed after 12 days. The FACS-BM contained 70–90 CFU-S per 10^3 cells. In accordance with the measured seeding efficiency of the FACS-BM cells to the spleen (data not shown), the concentration of FCS-S was calculated to be 50–107%. The appropriate number of FCS-BM cells were seeded onto the irradiated stromal cell layers, cultures terminated at various times and the adherent cells scraped off the flask and pooled with the suspension cells. One-fifth of each culture was injected into five irradiated mice to measure total CFU-S per culture. The results of one experiment are shown here, but we have obtained identical results in four other experiments.

with subsequent production of high numbers of CFU-S for many weeks¹. From these data we can conclude that an inoculum of normal bone marrow containing ~ 130 CFU-S is capable of colonizing a haematopoietic environment and maintaining self-renewal for at least 4–5 weeks. There does not seem to be appreciable stromal cell contribution to the irradiated stromal elements by the fresh normal bone marrow cells at this dose.

When various numbers of FACS-treated bone-marrow cells (FACS-BM cells) were seeded onto irradiated adherent layers and the cultures were terminated after 1,2 or 3 weeks to measure

Table 3 Generation of putative haematopoietic cell foci in irradiated marrow stromal cell layers

Input FACS-BM	Foci of putative haematopoietic cells (weeks)			
	1	2	3	4
5,000	+	++	—	—
1,250	+	++	—	—
312	+	+	+	—
78	—	+/-	+	+/-
20	—	+/-	+	+/-
0	—	—	—	—

Foci present in 60–100% random fields of view (++); 30–60% (+), 1–30% (+/-), no field of view (—). Irradiated marrow stromal cell layers were seeded with FACS-BM exactly as described in Table 2 legend. Cultures were examined weekly using a Leitz diavert inverted microscope. Foci of putative haematopoietic cells were classified according to the scheme above.

Table 4 CFU-S production in irradiated marrow stromal cell layers inoculated with reconstituted FACS-BM cells

Input	Input CFU-S	CFU-S per total culture (weeks)			
		1	2	3	4
NBM 5×10^5	130	34	240	174	132
Reconstituted FACS-BM 6.5×10^5	180	>250	>250	ND	185

Irradiated marrow stromal cell layers were established, seeded with cells and assayed weekly for total CFU-S per culture as described in Tables 1 and 2 legends. In one set of cultures, 5×10^5 normal bone marrow cells were seeded onto irradiated marrow stromal cell layers. For another set, the bone marrow was sorted through the FACS as described in Table 2 legend. After sorting, the FACS-BM was recombined with cells rejected at the metrizamide stage and during the FACS sorting to produce reconstituted FACS-BM cells, which were seeded onto irradiated marrow stromal cell layers.

the total CFU-S present in the growth medium and within the adherent layer, we consistently detected few or no CFU-S (Table 2). Apparently the FACS-BM CFU-S are neither maintained nor produced in such cultures, even when an inoculum of 700 CFU-S was used, equivalent to $\sim 3 \times 10^6$ normal bone marrow cells. Although these cultures were devoid of CFU-S, survival and production of maturing haematopoietic cells occurred. When the FACS-BM cells were plated onto the stromal cells, clonal foci of haematopoietic cells developed in the adherent layer. The number of foci was roughly proportional to the

number of FACS-BM cells introduced to the cultures and their sequential development from two or four cell foci into typical 'cobblestone' areas composed of hundreds of cells was observed. Furthermore, these foci were not stable and we observed the appearance, growth and disappearance of individual foci over a period of days (Table 3). Later than this, no foci were seen and the adherent layer became infiltrated randomly by macrophage-type cells. The cells in these foci are clearly not CFU-S; granulocyte-macrophage colony-forming cell assays (data not shown) only detected many macrophage cluster-forming cells. However, at the time of peak cobblestone formation the cell counts in the growth medium were often $>10^5$ per ml and the cells being produced consisted of blast cells, immature and maturing neutrophils and macrophages. Because of this, it is likely that the foci consist of maturing myeloid cells, although the identity of the blast cells is unknown.

From these data it is clear that normal bone marrow cells (containing limited numbers of CFU-S) can maintain haematopoiesis for some weeks, whereas FACS-BM (containing the same number of, or more, CFU-S) shows no ability to self-renew CFU-S or to establish long-term haematopoiesis *in vitro*. However, the FACS-BM cells can 'colonize' the stroma and form foci of cells of haematopoietic origin that undergo a limited amount of proliferation and development. The inability of FACS-BM cells to express self-renewal capacity does not result from the physical and mechanical stress of FACS sorting and the binding and removal of wheat-germ agglutinin as shown in Table 4.

Thus, other possibilities must be considered for the lack of growth of FACS-sorted CFU-S in irradiated long-term bone marrow cultures: (1) Irradiated stroma is defective in its ability to promote self-renewal of CFU-S. This seems unlikely because haematopoiesis can be established on the irradiated stroma by 5×10^5 normal bone marrow cells (which contain few stromal cells). (2) Removal of some accessory cell(s) important for the maintenance or control of CFU-S self-renewal. We are currently investigating this possibility. (3) Lack of feedback inhibition on differentiation. As FACS-BM cells differ from normal marrow cells in the absence of mature cells, it is possible that the mature cells somehow regulate the probability of self-renewal of CFU-S. (4) Finally, it is possible that the CFU-S are not the cells which repopulate haematopoiesis in long-term bone marrow cultures, that is, they are not the most primitive stem cells. Although FACS-purified CFU-S can promote 30-day survival of irradiated mice, no study has been made of the longer-term quality of the CFU-S⁶. The haematopoietic stem-cell compartment is clearly a heterogeneous population^{2,9} and certain elements of the CFU-S population (or even primitive stem cells that do not have spleen colony-forming capacity) may be discarded by the sorting procedure. Thus, although FACS-sorting seems to select for CFU-S with an average (or high) 'self-renewal' capacity *in vivo* in the short term⁶, the CFU-S may still not be representative of the stem cells.

These findings have obvious clinical importance. Recently, various methods for cleaning up human bone marrow cells before transplantation have been described, aimed at removing from the graft T cells^{10,11} responsible for provoking graft-versus-host disease. Treatment of bone marrow grafts in this way usually leads to alleviation of the disease, but also leads to a surprisingly high level of graft failure in some patients, for unknown reasons¹¹. Therefore, an understanding of why FACS-BM CFU-S do not self-renew *in vitro* may explain some of the problems associated with engraftment of human marrow *in vivo*. Our results indicate caution in the use of highly purified human haematopoietic progenitor cells for transplantation. Furthermore, the observation that CFU-S from FACS-BM do not self-renew *in vitro* provides an opportunity to investigate circumstances and conditions required for the self-renewal of haematopoietic stem cells.

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Expression of the *c-fms* proto-oncogene during human monocytic differentiation

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The McDonough strain of the feline sarcoma virus contains a transforming gene (*v-fms*) which contains partial nucleotide homology with proto-oncogenes encoding tyrosine kinases¹⁻⁴. One of the *v-fms*-encoded products, gp140^{fms}, is a cell surface transmembrane glycoprotein that may function as a growth factor receptor^{5,6}. Although *c-fms* transcripts have been detected in placental trophoblasts⁷⁻¹⁰ and normal human bone marrow¹¹, the role of the *c-fms* gene product is unknown. We now report that induction of monocytic, but not granulocytic, differentiation of human HL-60 leukaemic cells is associated with expression of *c-fms*, preceded by that of *c-myc* and *c-fos*. Because *c-fms* transcripts are also detectable in peripheral blood monocytes and in blasts from certain patients with myelomonocytic leukaemia, the *c-fms* gene product may play a role in monocytic differentiation.

The *c-fms* gene and the normal cellular homologue of the transforming gene of the FBJ murine osteosarcoma virus (*v-fos*) are transcriptionally active in the cells of mouse extra-embryonal membranes⁷⁻¹⁰. Expression of the *c-fms* gene was undetectable in a variety of postnatal mouse tissues⁷⁻⁹, whereas *c-fos* transcription was restricted to haematopoietic cells¹⁰. This specific pattern of transcriptional activity suggests that both *c-fos* and *c-fms* are involved in certain differentiation pathways^{7,9}. *c-fos* gene expression is associated with monocytic differentiation^{10,12,13} and *c-fms* transcripts have been detected in undifferentiated mouse haematopoietic cells¹², certain human tumours¹⁴ and normal human bone marrow¹¹. Moreover, the detection of *c-fms* sequences on chromosome 5 (ref. 15) and an association of the 5q⁻ syndrome with certain myelodysplastic syndromes¹⁶ and acute myelogenous leukaemia¹⁷ suggested that *c-fms* gene expression is related to haematopoietic cell differentiation.

We have used the human HL-60 promyelocytic cell line¹⁸ to study the role of *c-fms* expression during induction of haematopoietic cell differentiation. HL-60 cells mature morphologically and functionally towards granulocytes after induction with dimethyl sulfoxide (DMSO) or hexamethylene bisacetamide (HMBA)^{19,20}. In contrast, these cells can be induced to differentiate along a monocyte-macrophage pathway when treated with 12-*O*-tetradecanoylphorbol-13-acetate (TPA)²¹ and 1,25 dihydroxy-vitamin D₃ (1,25(OH)₂D₃)²². HL-60 cells were collected 24 and 72 h after addition of each of these inducers to monitor for *c-fms* expression. Although no *c-fms* RNA was detected in uninduced HL-60 cells (Fig. 1, lane 1), *v-fms* DNA hybridized with a 5-kilobase (kb) transcript detectable after 24 h of TPA and 1,25(OH)₂D₃ treatment. In contrast, *c-fms* RNA was not detected following induction of HL-60 cells with DMSO and HMBA. Similar results were obtained after

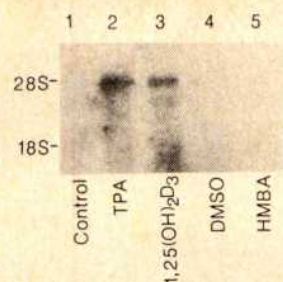


Fig. 1 Level of *c-fms* RNA during induction of HL-60 monocytic (TPA and 1,25(OH)₂D₃) and granulocytic (DMSO and HMBA) differentiation. HL-60 cells were grown as described previously¹⁸ in the presence of 3.3×10^{-8} M TPA (Sigma), 5×10^{-7} M 1,25(OH)₂D₃ (Hoffman-LaRoche), 1.25% DMSO or 4 mM HMBA for 24 h. Total cellular RNA was purified by the guanidine thiocyanate-caesium chloride method²⁹, analysed by electrophoresis of 15 µg RNA through 1% agarose-formaldehyde gels followed by Northern blot transfer to nitrocellulose³⁰. The 1.0-kb *Pst*I fragment of the *v-fms* gene was purified from the pSM3 plasmid⁴ by preparative gel electrophoresis and nick-translated to specific radioactivities of $\sim 5 \times 10^8$ c.p.m. µg⁻¹ DNA. The filters were pre-hybridized at 42 °C for 16–24 h in buffer consisting of 50% formamide, 5 × SSC, 0.1% SDS, 5 × Denhardt's and 200 µg ml⁻¹ salmon-sperm DNA. The RNA blots were then hybridized for 16–24 h at 42 °C with 2×10^6 c.p.m. nick-translated probe per ml hybridization buffer (same as the pre-hybridization buffer, with 1 × Denhardt's). After hybridization, the blots were washed twice in 2 × SSC, 0.1% SDS at room temperature for 60 min and then washed twice in 0.1 × SSC, 0.1% SDS at 55 °C for 30 min. The blots were then dried and exposed to X-ray film with an intensifying screen at -70 °C. Cytocentrifuge smears of HL-60 cells were examined after 3 days of continuous drug exposure for α-naphthyl acetate esterase (NSE) activity and nitroblue tetrazolium (NBT) reduction³¹. Adherence to the tissue culture flask was also monitored on day 3. Control HL-60 cells: 0% adherence, 13.3 ± 1.5% NSE positive, 7.3 ± 1.5% NBT positive; TPA-treated cells: 84.7 ± 1.8% adherence, 71.3 ± 2.9% NSE, 13.3 ± 2.4% NBT; 1,25(OH)₂D₃-treated cells: 0% adherence, 86.0 ± 5.6% NSE, 99.3 ± 0.8% NBT; DMSO-treated cells: 0% adherence, 19.3 ± 3.3% NSE, 94.3 ± 1.9% NBT; HMBA-treated cells: 0% adherence, 0% NSE, 84.5 ± 3.4% NBT. (Results are expressed as mean ± s.d. of four determinations).

72 h treatment with each inducer and *c-fms* transcripts were not detected (data not shown) after TPA exposure of an HL-60 blast subclone²³ that does not undergo differentiation following treatment with inducers of monocyte or granulocyte differentiation. Taken together, these findings suggest that *c-fms* is induced when the HL-60 cells mature along the monocyte-macrophage pathway and that TPA is not a general inducer of *c-fms* gene transcription.

The temporal relationship between the addition of TPA and *c-fms* expression is shown in Fig. 2a. The *c-fms* transcripts appear 6 h after TPA addition, whereas maximum levels of *c-fms* RNA were present following 24–48 h of induction. These findings contrast with *c-myc* expression which, as shown previously²⁴, was not detected 24 h after TPA induction (Fig. 2a). Similar declines in *c-myc* transcripts occur following TPA treatment of HL-60 cells¹⁹, but these changes in *c-fms* and *c-myc* RNA during induction of HL-60 monocytic differentiation were not associated with altered *N-ras* or actin gene expression (Fig. 2a). We have also monitored *c-fms* expression during 5 days of TPA or 1,25(OH)₂D₃ treatment and find that *c-fms* RNA remains detectable following induction with 1,25(OH)₂D₃ (Fig. 2b). Similar results have been obtained after induction with TPA. Moreover, TPA treatment of HL-60 cells for 20 min is sufficient for commitment along the monocyte differentiation pathway¹³. The *c-fms* transcripts, however, were not detectable 24 h after a similar 20-min TPA exposure (data not shown).

Within 1 h of treating HL-60 cells with TPA, *c-fos* messenger RNA is detectable¹³. We have also examined the relationship

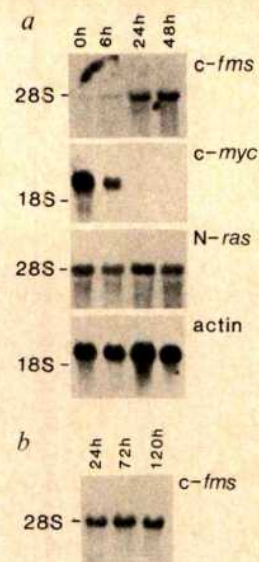


Fig. 2 Temporal relationship of *c-fms* expression during induction of HL-60 cells by TPA and 1,25(OH)₂D₃. **a**, HL-60 cells were treated with 3.3×10^{-8} M TPA for 6, 24 and 48 h. RNA (15 µg) was then hybridized to the ³²P-labelled nick-translated cDNA probes. The 1.6-kb *Cl*AI/*E*coRI fragment of the human *c-myc* 3' exon was purified from the pMC41-3 RC plasmid³². The 1.5-kb *p*52C-*E*coRI fragment of the HL-60 *N-ras* gene was purified from a reconstructed pBR322 plasmid³³. The 2.0-kb *Pst*I fragment of the chicken β-actin gene was purified from the pA1 plasmid³⁴. All methods are described in Fig. 1 legend. **b**, HL-60 cells were treated with 5×10^{-7} M 1,25(OH)₂D₃ for 24, 72 and 120 h. RNA (20 µg) was then hybridized to the ³²P-labelled *v-fms* probe.

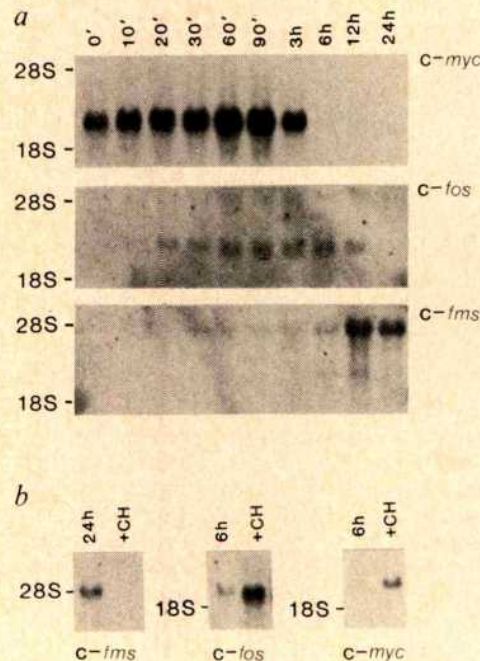


Fig. 3 **a**, Relationship between expression of *c-fms*, *c-fos* and *c-myc* in TPA-induced HL-60 cells; **b**, effect of protein synthesis inhibition on *c-fms*, *c-fos* and *c-myc* expression. **a**, HL-60 cells were treated with 3.3×10^{-8} M TPA and collected at the times indicated. Total cellular RNA (20 µg) and specific transcripts were detected by hybridization to ³²P-labelled complementary DNA probes as described in Figs 1, 2 legends. The 1-kb *Pst*I fragment of the *v-fos* gene was purified from the pfos-1 plasmid³⁵. **b**, HL-60 cells were treated for 6 or 24 h with 3.3×10^{-8} M TPA alone and in combination with cycloheximide (+CH). Procedures for RNA preparation and analysis were as described in Figs 1, 2 legends except that 20 µg total RNA were used in each lane.

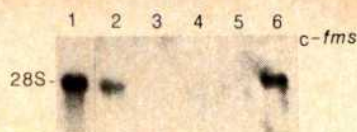


Fig. 4 *c-fms* gene transcripts in human monocytes and blasts from patients with myelomonocytic leukaemia. Peripheral blood monocytes and blasts from patients with myelomonocytic leukaemia were prepared as described previously²⁹. The ³²P-labelled *v-fms* probe was then hybridized to RNA (20 µg) obtained from these cell preparations: lane 1, normal peripheral blood monocytes (100% adherent cells); lane 2, TPA-induced HL-60 cells (24 h); lanes 3–6, blast preparations from four patients with myelomonocytic leukaemia (leukaemic blast composition by morphology: lane 3, 80%; lane 4, 79%; lane 5, 73%; and lane 6, 85%). All methods as described in Fig. 1 legend.

between changes in *c-myc*, *c-fos* and *c-fms* RNA during 24 h of TPA induction. The *c-myc* RNA level increased fourfold by 90 min after TPA addition (determined by optical densitometry of the autoradiograph) and then declined to nearly undetectable levels by 6 h (Fig. 3a). As reported previously¹³, *c-fos* transcripts accumulated within 20–30 min following induction, reached a maximum level at 90 min and declined thereafter. In contrast, *c-fms* expression occurred later, 6 h after TPA induction, and increased by 24 h (Fig. 3a). Clearly, the decrease in *c-myc* RNA and the increase of *c-fos* RNA both precede induction of *c-fms* expression.

We have extended our comparison of *c-myc*, *c-fos* and *c-fms* expression by monitoring the effects of cycloheximide on the level of these transcripts. Although *c-fms* expression was detectable in the 6-h TPA-treated cells, concurrent cycloheximide treatment inhibited the induction of *c-fms* RNA. Similar results were obtained after 24 h of TPA and cycloheximide treatment (Fig. 3b). In contrast, cycloheximide increased expression of both *c-myc* and *c-fos* (Fig. 3b). Thus, cycloheximide potentiates expression of the *c-myc* and *c-fos* genes known to be induced by mitogens^{13,25}, whereas our results suggest that induction of *c-fms* RNA requires new protein synthesis.

The differential sensitivity of *c-fos* and *c-fms* expression to cycloheximide, as well as the possible sequencing of these genes, may be related to specific functions of the gene products during monocyte proliferation and differentiation. Transcription of the *c-fos* gene, but not the *c-fms* gene, occurs within minutes of stimulating quiescent mouse fibroblast 3T3 cells with TPA²⁶. The early increase in both *c-myc* and *c-fos* mRNA, but not *c-fms* mRNA, has also been reported in TPA-treated quiescent fibroblasts and suggests that the *c-myc* and *c-fos* gene products are necessary for the mitogenic response²⁶. Conversely, transfection of F9 teratocarcinoma cells with an exogenous *c-fos* gene induces expression of only certain genes in the differentiation programme, thus indicating that maturation to a terminal stage of differentiation requires the expression of additional genes not regulated by *c-fos*²⁷. Thus, in TPA-treated HL-60 cells, the *c-fos*-encoded nuclear protein may play a dual role in both cell growth and initiation of differentiation. In contrast, the *c-fms* gene product may function in terminal differentiation along the monocyte-macrophage pathway.

Therefore, we have monitored the level of *c-fms* transcripts in circulating human monocytes and in blasts from four patients with myelomonocytic leukaemia (Fig. 4), and found that peripheral blood monocytes (Fig. 4, lane 1) and one of four leukaemic blast preparations had detectable levels of *c-fms* RNA (Fig. 4, lanes 3–6). The size of the transcript detected in leukaemic cells was identical to that observed in TPA-induced HL-60 cells (Fig. 4, lane 2) and in circulating monocytes. Furthermore, the 5-kb transcript was also detectable after TPA-induction of the human U-937 cell line to macrophages (data not shown). In contrast, *c-fms* RNA was undetectable in human granulocytes²⁸, peripheral blood T-cells, K562 erythroleukaemia

cells, B-cell lines (Daudi and JY), a T-cell line (MOLT-4) and AG1522 diploid fibroblasts.

Murine myeloid monocytic leukaemia WEHI-3B cells contain two *c-fms* transcripts of 4.1 and 8.4 kb (ref. 9). A smaller 3.7-kb *c-fms* transcript has been detected in mouse placental tissue⁸ and a transcript of the same size has been detected in a trophoblastic cell line (BeWo) derived from a gestational choriocarcinoma of the human fetal placenta⁹. In contrast, the results of the present study suggest that the *c-fms* transcript is 5 kb long in HL-60 and U-937 cells induced along a monocyte lineage, monocytes and certain leukaemic blasts. These differences in transcript size may be related to the physiological function of the *c-fms* encoded products in gestational and haematopoietic tissues.

Thus, expression of the *c-fms* gene has been observed in haematopoietic cell lines induced along the monocyte differentiation pathway. Furthermore, the detection of the *c-fms* transcripts in circulating monocytes, but not granulocytes or lymphocytes, implies that the gene product is involved specifically in the process of monocyte-macrophage differentiation. The localization of *c-fms* to chromosome 5 (ref. 15) is of interest in view of a specific deletion involving this region in refractory anaemia¹⁶ and acute myelogenous leukaemia¹⁷. Unregulated transcription of the *c-fos* gene might lead to uncontrolled proliferation²⁶. In view of the possible sequencing of proto-oncogene expression during monocytic differentiation, it would be of interest to determine whether the *c-fms* gene product regulates the expression of *c-myc* or *c-fos* genes. Thus, a decrease in the production of the *c-fms* gene product could lead to defects not only in the control of cell differentiation along the monocyte lineage, but also in cell proliferation.

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Functional expression of a microinjected E_α^d gene in C57BL/6 transgenic mice

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The class II major histocompatibility antigens, I-A and I-E, have been detected on the surface of certain immunocompetent cells, including B lymphocytes and monocytes¹⁻³. These molecules are involved in cell-cell interactions in the immune responses^{4,5}. Each class II antigen consists of two subunits, α and β chains, and the genes encoding these subunits have been well characterized at the molecular level⁶⁻⁹. To analyse the regulatory mechanism of E_α gene expression and the role of the I-E antigen in the regulation of the immune responses, we have produced transgenic mice by microinjecting cloned E_α^d genes into fertilized eggs of C57BL/6 mice of *b* haplotype. This strain of mouse carries a deletion in the upstream (5') region of the E_α gene covering the transcriptional promoter and, therefore, does not express this gene¹⁰. Interestingly, this genetic defect of the E_α gene is accompanied by the inability of the host mouse to respond to a certain set of antigens, phenomena generally termed *Ir* gene control^{11,12}. We report here that the E_α^d genes are expressed in these transgenic mice to form the I-E $^\alpha E_\beta$ antigen on the surface of B lymphocytes and monocytes and that these I-E antigens are functional in terms of the induction of a mixed lymphocyte reaction and the restoration of immune responsiveness to poly(L-glutamic acid-L-lysine-L-phenylalanine) (GL-Phe).

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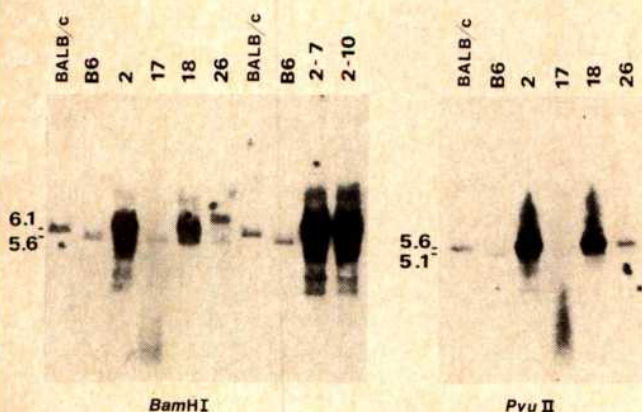


Fig. 1 Southern blot analysis of the E_α gene in transgenic mice³². DNAs (5 μ g) from liver of BALB/c, C57BL/6 or transgenic mice were digested with *Bam*HI or *Pvu*II, electrophoresed on a 0.5% agarose gel, transferred to a nitrocellulose filter and hybridized with the ³²P-labelled 2.6-kb *Sal*I fragment which includes the first exon of the E_α gene. The host C57BL/6 mice carry a deletion in this region³². Mice 2, 17, 18 and 26 were born from ova injected with E_α^d DNA. Mouse 17 did not contain injected genes. The offspring (nos 2-7 and 2-10) of mouse 2 were produced by mating mouse 2 with a female C57BL/6 mouse. DNAs from mice 2-7 and 2-10 were also analysed by *Bam*HI digestion. Sizes of DNA fragments are in kilobases.

Approximately 200 copies of the 14-kilobase (kb) *Sac*II/*Xho*I fragment containing the entire E_α^d gene sequence were microinjected into each fertilized egg of the C57BL/6 mouse. In total, 30 mice were born. These mice were partially hepatectomized and the DNA isolated from the livers was analysed by Southern blotting to determine whether the injected E_α^d gene is retained in the host DNA. Because of the aforementioned deletion, BALB/c DNA yielded a DNA fragment 0.5 kb larger than that of C57BL/6 DNA when these DNAs were digested either with *Bam*HI or *Pvu*II and the digests analysed with a probe covering the 5' end of the E_α gene (see Fig. 1). This allows us to distinguish the injected E_α^d gene from the endogenous defective E_α^b gene present in the host C57BL/6 mouse. As expected, DNA from transgenic mice gave the 5.6-kb *Bam*HI or 5.1-kb *Pvu*II band characteristic of the C57BL/6-derived, defective E_α^b gene (Fig. 1). In addition, DNA from transgenic mice 2 and 18 gave the 6.1-kb *Bam*HI band and 5.6-kb *Pvu*II band diagnostic of the E_α^d gene, indicating that these mice have retained the injected BALB/c E_α^d gene. DNA from the third transgenic mouse, no.

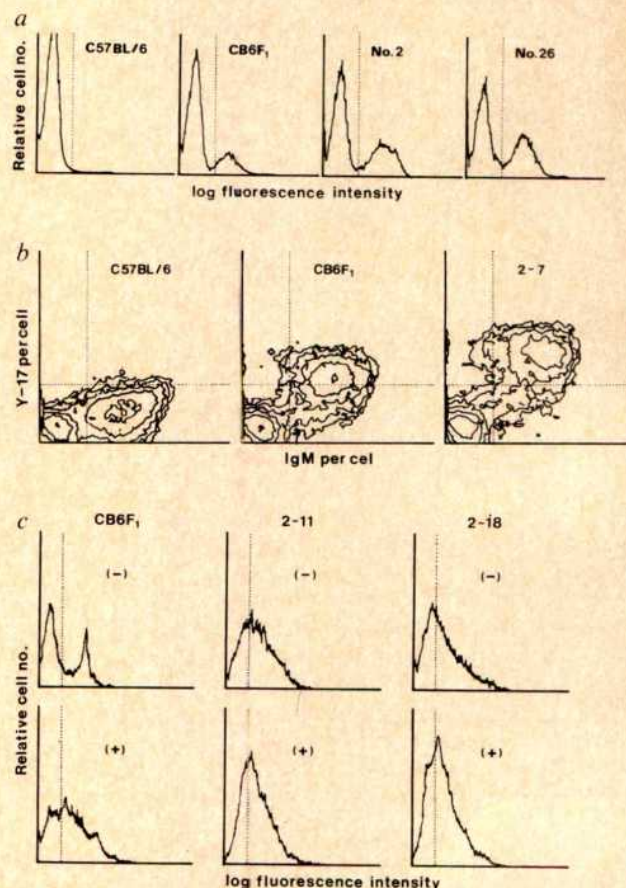


Fig. 2 Expression of the I-E $^\alpha E_\beta$ antigens on lymphocytes and peritoneal monocytes of the transgenic mice. *a*, Mononuclear cells were prepared from peripheral blood of CB6F₁ and C57BL/6 mice and of mice 2 and 26 on a Ficoll-Hypaque gradient. Cells were incubated with the biotininated Y17 antibody, followed by fluoresceinated avidin and analysed with a fluorescence-activated cell sorter, FACS-440 (Becton-Dickinson). *b*, Spleen lymphocytes from CB6F₁, C57BL/6 mice and an offspring (no. 2-7) of mouse 2 were incubated with the Y17 antibody and fluorescein isothiocyanate (FITC)-conjugated anti-mouse μ chain, followed by phycoerythrin-conjugated avidin and analysed with a cell sorter. *c*, Peritoneal exudate cells were prepared from CB6F₁ mice and from the offspring of mouse 2 which had been injected intraperitoneally with 1 ml 3% proteose peptone 3 days earlier. Cells (1×10^6 in 1 ml) were incubated in a 24-well plastic plate at 37 °C and non-adherent cells were removed by washing with Hank's balanced salt solution. Adherent cells were cultured with or without 50% (v/v) supernatant of Con A-stimulated spleen cells for 2 days. This supernatant contains, respectively, 100 U ml⁻¹ and 650 U ml⁻¹ γ -interferon and interleukin-2 activity. The cells were then stained with the Y17 antibody and analysed with a cell sorter.

26, also displayed the 5.6-kb *PvuII* band but the size of the corresponding *Bam*HI fragment was somewhat greater than 6.1 kb. This discrepancy may result from loss of the 5' *Bam*HI site. From an approximate calculation based on the intensities of the E_α^d bands, mice 2 and 18 seem to have at least 10 copies of the E_α^d gene. These E_α^d genes were transmitted to a fraction of offspring, two examples of which (nos 2-7 and 2-10) are shown in Fig. 1. In the following studies, both the original transgenic mice and their offspring were used.

Expression of the E_α gene in the transgenic mice was examined by the surface staining of peripheral blood mononuclear cells and splenic lymphocytes using a monoclonal anti-I-E antibody, Y17 (ref. 13). Because this antibody can recognize combinations of E_α and E_β molecules of various haplotypes, including $E_\alpha^d E_\beta^b$, we could examine whether the product of the microinjected E_α^d gene can pair with the endogenous E_β^b chain and be displayed on the cell surface as the hybrid $E_\alpha^d E_\beta^b$ molecule. As shown in Fig. 2a, about one-third of the peripheral blood lymphocytes from mice 2 and 26 could be stained with Y17, indicating that the lymphocytes of the transgenic mice expressed the hybrid I-E^{d/b} antigen. The fluorescence intensity of the Y17-positive cells of the transgenic mice seems to be significantly higher than that of a CB6F₁ (C57BL/6 × BALB/c F₁) mouse. As expected, the I-E^{d/b} antigen was mainly expressed on B lymphocytes bearing immunoglobulin M (IgM) molecules on the cell surface (Fig. 2b). We also analysed E_α^d gene expression on the monocytes of the transgenic mice. Peritoneal monocytes of the transgenic and CB6F₁ mice, as the control, were cultured for 48 h with or without the culture supernatant of concanavalin A (Con A)-stimulated murine spleen cells containing 50 U ml⁻¹ of γ -interferon and stained with the Y17 antibody. As expected, the I-E^{d/b} molecules were detected on a very small fraction of the unstimulated cells and on most of the stimulated cells of the CB6F₁ mouse. In contrast, most of the peritoneal monocytes from the transgenic mice expressed the I-E^{d/b} antigen in the absence of the Con A supernatant and the addition of the supernatant to the culture media did not increase the level of I-E^{d/b} expression. The reason for this constitutive expression is unknown, but it may be related to the fact that the transgenic mice examined (nos 2-11 and 2-18) carry a large copy number (>20) of E_α^d genes. In any case, it is interesting that not only the injected E_α^d genes but also the endogenous E_β^b gene seem to be constitutively expressed in these mice.

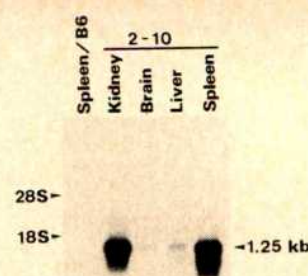


Fig. 3 Northern blot analysis of E_α mRNA from various tissues of a transgenic mouse. Total RNA was phenol-extracted from spleen, liver, kidney and brain of a transgenic mouse and adsorbed to oligo(dT)-cellulose; 2 μ g poly(A)⁺ RNA from spleen and 5 μ g poly(A)⁺ RNA from liver, kidney and brain were denatured, electrophoresed on a 1% formaldehyde-containing agarose gel, transferred to a nitrocellulose filter and hybridized with a ³²P-labelled 3.5-kb *SalI* fragment containing exons 2, 3 and 4 of the E_α gene as described previously³³. As a control, 2 μ g mRNA from C57BL/6 spleen was also analysed. Sizes of RNA molecules are shown in kilobases.

To examine the transcription of the microinjected E_α^d gene, we performed a Northern blot analysis of the E_α transcript in various tissues from a transgenic mouse, no. 2-10. Figure 3 shows that detectable amounts of E_α messenger RNA of 1.25 kb were observed in poly(A)⁺ RNA from all analysed tissues of the transgenic mouse. The microinjected E_α^d gene seemed to be transcribed at a low level in liver and brain cells. Although kidney cells of a transgenic mouse contained a relatively large amount of E_α mRNA, similar amounts of E_α transcripts were also observed in BALB/c kidneys (data not shown). The class II antigens are known to be expressed on B lymphocytes and macrophages in lymphoid organs^{1-3,14}, liver Kupffer cells^{15,16}, brain astrocytes¹⁶, kidney pelvis cells¹⁵ and blood vessel endothelial cells¹⁷. The observed transcripts of the E_α gene in tissues of the transgenic mouse may be due to these cells. Recently, a rabbit β -globin gene was reported to be expressed in skeletal muscle and testis but not in erythroid cells in transgenic mice¹⁶. Immunoglobulin μ heavy-chain genes were also expressed in heart as well as in lymphocytes¹⁹. We cannot

Table 1 I-E^{d/b} antigen in a transgenic mouse is functional in terms of induction of a mixed lymphocyte reaction and restoration of the response to GL-Phe

	Stimulator cells				Antigens			
	(-)	C57BL/6 (A ^b E ⁻)	Transgenic (A ^b E ^{d/b})	CB6F ₁ (A ^{b/d} E ^{b/d})	(-)	GL-Phe	KLH	PPD
C57BL/6	5,767 (±999)	7,233 (±1,149)	25,622 (±1,459)	90,557 (±15,417)	6,445 (±1,268)	6,326 (±837)	6,373 (±818)	47,296 (±13,307)
Transgenic	—	—	—	—	4,434 (±438)	15,228 (±4,612)	5,846 (±306)	20,314 (±5,061)
CB6F ₁	—	—	—	—	5,530 (±865)	25,363 (±1,067)	5,160 (±1,580)	58,340 (±22,137)

2.5 × 10⁵ responder lymph node cells from normal C57BL/6 mice were stimulated with 1 × 10⁶ mitomycin C (MMC)-treated cells from C57BL/6, CB6F₁ or transgenic mice in 0.2 ml complete culture medium in a microtitre plate (Falcon 3072, Becton-Dickinson) for 4 days. Complete culture medium consisted of RPMI 1640 culture medium, 10% heat inactivated fetal calf serum (FCS), 5 × 10⁻⁵ M 2-mercaptoethanol, 100 mM HEPES, 100 U ml⁻¹ penicillin, 100 μ g ml⁻¹ streptomycin and 2 mM glutamine. MMC treatment of stimulator cells: 5 × 10⁶ spleen cells were incubated in 1 ml complete culture medium in the presence of 50 μ g MMC at 37 °C in 5% CO₂ in air; after 30 min, cells were washed three times with Hank's balanced salt solution. Cells were pulsed with 1 μ Ci ³H-TdR for the final 16 h of culture. Cells were collected on a filter paper with a semi-automatic cell harvester (Labo Mash Science, Tokyo) and the uptake of ³H-TdR was measured by standard scintillation counting. The proliferative responses of responder cells are expressed as the uptake of ³H-thymidine (mean ± s.d. of triplicate cultures). The antigen-specific T-cell proliferative response was assayed according to the methods of Corradin *et al.*³¹. C57BL/6 mice (three in one group), CB6F₁ mice (three in one group) and a transgenic mouse were immunized with 50 μ g GL-Phe (given by Dr R. Schwartz, NIH) and emulsified in the complete Freund's adjuvant. After 1 week, draining lymph nodes were removed and single-cell suspensions made. Cells were cultured in complete culture medium containing 10% heat-inactivated horse serum instead of FCS in the presence of 200 μ g ml⁻¹ GL-Phe, 100 μ g ml⁻¹ KLH or 50 μ g ml⁻¹ purified protein derivative (PPD) from *Mycobacterium tuberculosis* (Mitui Pharmaceuticals, Tokyo). Cells were cultured for 4 days and the proliferative response assayed as described above.

exclude the possibility that the microinjected E_d^d genes are expressed in inappropriate cells of the transgenic mouse. To assess this possibility more precisely, further studies, including *in situ* RNA hybridization using tissue samples, are required.

Finally, we examined whether the newly expressed I-E^{d/b} antigen has immunological functions. Spleen cells of the transgenic mice could induce a significant proliferation of C57BL/6 lymphocytes (Table 1), suggesting that the I-E^{d/b} antigens of the transgenic mice could be successfully recognized as allo-antigens by the T lymphocytes from C57BL/6 mice. It is known that mouse strains of *H-2b*, *s*, *f* and *q* haplotypes do not respond to certain antigens such as GL-Phe, GL-Leu and pigeon cytochrome *c* (*Ir* gene phenomena)²⁰⁻²²; this unresponsiveness is correlated with the defect in I-E antigen expression in these strains²³. We therefore used responsiveness to GL-Phe as another criterion for the functional expression of the I-E^{d/b} antigens in the transgenic mice, and attempted to demonstrate directly that the *I-E* gene defect is the cause of the unresponsiveness. For this purpose, lymph node cells from mice primed with GL-Phe in complete Freund's adjuvant were assayed for a proliferative response to GL-Phe and, as a control, to keyhole limpet haemocyanin (KLH). We found that GL-Phe could induce significant proliferation of the lymphocytes from CB6F₁ mice (positive control) and from the transgenic mouse but not of lymphocytes from C57BL/6 mice (negative control) (Table 1). The proliferation was specific to the priming antigen (GL-Phe) because no proliferation above the background level was observed in the presence of KLH. These results suggest strongly that the product of the microinjected E_d gene is expressed on the surface of the antigen-presenting cells of the host mouse (C57BL/6) in such a way as to allow the restoration of responsiveness to the antigen GL-Phe.

In the past, several groups have successfully introduced cloned genes for major histocompatibility antigens into cultured cell lines²⁴⁻²⁶, and these transformants have been used to study antigen recognition by T lymphocytes²⁷⁻³⁰. However, this approach is not suitable for *in vivo* analysis of the roles of the major histocompatibility antigens in the generation of the diversity of T-lymphocyte repertoires. To date, this latter type of analysis has been carried out by using H-2 congenic mice, chimaeric mice and H-2 mutant mice. In the present study, we produced transgenic mice which express functional I-E antigens by microinjecting the cloned E_d^d DNA into fertilized eggs of C57BL/6 mice. These transgenic mice have several advantages over the aforementioned mice: (1) the transferred, cloned gene is well characterized; (2) a cloned gene can be modified by *in vitro* mutagenesis or exon shuffling; (3) it is possible to establish new strains carrying the E_d^d gene by crossing these mice with other B6 or B10 congenic mice. Thus, these transgenic mice should be a powerful tool for the analysis of the effect of well-defined class II genes on immune responses.

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Neutralization of human T-lymphotropic virus type III by sera of AIDS and AIDS-risk patients

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Human T-lymphotropic virus type III (LAV, HTLV-III)^{1,2} is aetiologically linked to acquired immune deficiency syndrome (AIDS) and persistent general lymphadenopathy (PGL)²⁻⁷. Specific radioimmunoassays (RIA), enzyme-linked assays, immunofluorescence assays (IFA) and immunoblotting techniques are being used widely to detect serum antibodies to HTLV-III in infected patients and in those at risk of infection⁴⁻⁷. However, these assays do not functionally identify those antibodies that neutralize the infectivity of the virus. We have used three methods of titrating serum neutralizing factors: inhibition of syncytium induction, neutralization of envelope pseudotypes of vesicular stomatitis virus (VSV) and reduction of infectivity of HTLV-III for a cell line permissive to virus replication. We report here that sera from subjects in various disease categories possess only low-level neutralizing activity, even when antibodies to viral membrane antigens are present in high titre. Envelope pseudotypes prepared from four HTLV-III isolates made in three different countries are equally sensitive to neutralization by positive sera, including sera from patients yielding two of the virus isolates.

To examine neutralizing properties of sera containing anti-HTLV-III, we initially determined whether such sera would inhibit the induction of syncytia or would neutralize VSV(HTLV-III) pseudotypes. When uninfected cells bearing HTLV-III receptors are mixed in excess with virus-producing cells, large multinucleated syncytia form within 6 h and are an indicator of viral envelope glycoprotein activity⁸. From previous studies⁹⁻¹² of HTLV-I and HTLV-II, we would expect antibodies binding to the functional epitopes of the viral envelope antigen both to inhibit syncytium formation and to neutralize the infectivity of pseudotypes.

Table 1 shows the mean titres of sera from patients naturally infected with one of the three known HTLV strains. As expected, sera containing antibodies to HTLV-I and HTLV-II specifically inhibited syncytium formation induced by these viruses and also

showed high titres of neutralizing activity specific for the respective VSV pseudotypes. In contrast, sera containing anti-HTLV-III showed negligible neutralizing activity even though the antibody titre for IFA antibody to cell-surface HTLV-III antigens was as high or higher than the IFA titres to HTLV-I and HTLV-II membrane antigens displayed by sera containing antibodies to the respective viruses. None of the 12 sera containing anti-HTLV-III showed significant syncytium inhibiting activity; there was slight inhibition of pseudotype plaques by six of the sera, but none had a neutralizing titre greater than 1:50. When sera containing high-titre neutralizing activity to HTLV-I or HTLV-II were tested for neutralization of VSV(HTLV-III), no cross-reaction was detected.

Because the sera containing anti-HTLV-III were from patients with symptoms of the AIDS-related complex, we sought to determine whether asymptomatic, yet seropositive, individuals have stronger neutralizing activity. The results of VSV(HTLV-III) neutralization tests on 97 positive sera (79 from the United Kingdom⁷ and 18 from Africa¹³) are shown in Table 2, categorized according to the clinical status of the patient, or his or her origin. At 1:10 dilution, only 15% of the sera exhibited neutralization of 80% or more VSV(HTLV-III) plaque-forming units (PFU), and these were mainly British or African patients with PGL. However, when a reduction of 40% PFU was taken as a positive score, then the sera of 69% patients from all clinical groups were found to contain neutralizing factors. The neutralizing activity was completely absorbed by protein A of *Staphylococcus aureus* (data not shown), indicating that it was a property of serum immunoglobulins.

The pseudotype neutralization tests had been performed using the American HTLV-IIIB isolate growing in HT-H9 cells¹⁴ as a source of envelope antigen for the VSV pseudotypes. As genomic polymorphisms have been observed, including variation in the putative *env* gene¹⁵, it seemed plausible that the very weak neutralization observed with British and African sera reflected divergence of envelope antigenicity resulting from the spread of HTLV-III through different host populations. Therefore, we prepared VSV(HTLV-III) pseudotypes with the envelope antigens of four reportedly independent HTLV-III isolates, and titrated neutralizing activity in selected sera including two (Bru and Arg) from patients from whom virus isolates (LAV-1 and CBL-1 respectively) had been made. Serum samples from the British patient Arg were available from before and after the date of biopsy leading to isolation of CBL-1.

Table 3 shows that the sera tested had similar neutralizing titres for VSV pseudotypes with the envelopes of French, British and American HTLV-III isolates. The sera did not have significantly stronger neutralizing activity for their homologous virus isolate. Thus, the relatively low neutralizing titres of sera containing anti-HTLV-III would not seem to be directly attributable to strain variation. When four positive sera were combined, the neutralizing titre was no higher than that of the strongest serum in the pool. Furthermore, an American commercial immunoglobulin G preparation, pooled from several hundred sera selected for hepatitis B virus antibodies and possessing high anti-HTLV-III by RIA, contained barely detectable neutralizing antibodies (Table 3).

It may be argued that the low neutralizing titres of sera containing anti-HTLV-III assayed by syncytium inhibition and by pseudotype inactivation do not reflect the sensitivity to neutralization of HTLV-III itself. We therefore compared the effect on HTLV-III infectivity of four sera previously found to have high IFA titres and relatively strong, weak or undetectable neutralizing activity for VSV(HTLV-III) pseudotypes. Table 4 shows that the neutralizing titres for infectious units of HTLV-III and of VSV(HTLV-III) were closely parallel, although inactivation of HTLV-III may be slightly more sensitive. Low-titre neutralizing activity of human sera to HTLV-III is also being reported by others^{16,17,36,37}. Thus, the rapid and quantitative VSV pseudotype neutralization test serves as an accurate estimate of the protective effect of sera in neutralizing HTLV-III infectivity.

Table 1 Mean titres of human sera from subjects infected with HTLV types I, II or III assayed by different methods

Virus	No of sera	Competitive RIA	Membrane IFA	Syncytium inhibition	Pseudotype neutralization
HTLV-I	9	1,216	2,250	180	3,257
HTLV-II	4	774	2,500	825	3,750
HTLV-III	12	1,272	2,847	<10	<10

Human sera known to be positive by RIA for antibodies to HTLV-I, -II or -III were titrated after heat inactivation (56 °C for 30 min) by methods described previously^{7,10-12}. Values are the geometric means of the reciprocals of the highest serum dilution giving >50% inhibition radioimmunoassay (RIA), >80% fluorescing cells (IFA) and >80% reduction of nuclei in syncytia or of PFU of VSV pseudotype. For both syncytial and pseudotype assays of HTLV-I and HTLV-II, Human osteosarcoma (HOS) cells were used as indicator cells¹¹. JM cells served as indicator cells for syncytial assays of HTLV-III⁸, adherent CEM cells pretreated with 25 µg ml⁻¹ DEAE-dextran were used for assay of VSV(HTLV-III) pseudotypes, and were subsequently overlaid with HOS cells and then agar medium, as described previously⁸.

Table 2 Neutralization of VSV(HTLV-III) pseudotype by sera of subjects positive by RIA

Serum donor	No of sera	No of sera reducing VSV(HTLV-III) plaques by	
		>40%	>80%
AIDS, KS	6	6	1
AIDS, OI	6	2	0
PGL only	13	12	4
PGL plus minor OI	4	4	2
Contacts of AIDS or PGL	9	7	2
Symptomatic homosexuals	8	6	1
Asymptomatic			
homosexuals	11	6	0
Haemophiliacs	22	11	0
Africans with KS or PGL	18	13	4
Total seropositives	97	67	14
Seronegative controls	20	0	0

Heat-inactivated sera were incubated at 1:10 dilution with 5×10^2 PFU VSV(HTLV-III) for 1 h at 33 °C and surviving PFU were plated on adherent CEM cells as described for Table 1. Positive sera taken in the United Kingdom were selected from a previous study⁷, as were the uninfected control sera. Africans with Kaposi's sarcoma (KS) or PGL were Zambians¹³. Patients with AIDS were identified clinically as opportunistic infection (OI) and/or KS according to the US Centers for Disease Control definition. Patients with PGL had enlarged lymph nodes (>1 cm) in two or more extra-inguinal sites persisting for more than 3 months according to the Centers for Disease Control definition. Two patients had oral candidiasis, one developed a lung abscess following aspiration during an epileptic fit. One patient developed AIDS 12 months after the serum specimen was taken. AIDS and PGL contacts were the regular sexual partners for at least 6 months before diagnosis of AIDS or PGL in their partner. All were well and without lymphadenopathy. Symptomatic homosexuals included six patients with recent viral-like illnesses accompanied by lymphadenopathy (4), pharyngitis (2) and an unexplained transient erythematous macular rash (1), one patient with generalized lymphadenopathy and malaise of <3 months' duration and one patient with a streptococcal pneumonia. Asymptomatic homosexuals were men attending the clinic with no systemic symptoms or generalized lymphadenopathy. Haemophiliacs had received regular clotting factor replacement therapy. Neutralization tests were performed and scored 'blind', that is, without knowledge of RIA titres or clinical status of the patient.

The low titres of virus neutralizing antibodies in subjects infected with HTLV-III contrasts with the type-specific, high-titre human humoral response to HTLV-I and HTLV-II⁹⁻¹², and also differs from the immune response of animals naturally infected with avian¹⁸, feline¹⁹ and bovine²⁰ leukaemia viruses. HTLV-III is morphologically more closely related to the lentivirus family of retroviruses, reportedly exhibiting weak cross-reaction of antibody to the core antigen of equine infectious anaemia virus (EIAV)²¹, and partial genomic homology to visna virus (VV)²². Strongly neutralizing sera are commonly detected in animals infected with EIAV^{23,24} and VV^{25,26}, although neutralizing activity has not been found in goats infected with

Table 3 Neutralization of VSV pseudotypes with envelopes of different HTLV-III isolates including matched sera

Serum donor	LAV-1 (France)		Titre neutralizing VSV(HTLV-III) plaques (%)				IIIA (USA)	
	40	80	CBL-1 (UK)		IIIB (USA)		40	80
Bru (LAV-1)	50	10	50	10	50	10	50	10
Arg (CBL-1) October 1983	50	<10	25	<10	50	<10	50	<10
Arg (CBL-1) September 1984	50	<10	50	<10	50	<10	50	<10
Neb	50	10	50	10	50	10	50	10
Sel	250	50	250	50	250	50	250	50
Pooled IgG (USA)	50	<10	50	<10	50	<10	50	<10
Uninfected control	<10	<10	<10	<10	<10	<10	<10	<10

VSV pseudotypes were prepared with four isolates of HTLV-III and plated on adherent CEM cells (Table 1) following incubation with the sera indicated. The CBL-1 isolate was made from a lymphomatous lymph node biopsy taken from AIDS patient Arg in June 1984, 8 months after the first serum sample and 3 months before the second serum sample assayed (A.G.D., A. Horwich, M. G. Peckham and R.A.W., unpublished data); this patient was a homosexual living in the United Kingdom with a single sexual partner who was also seropositive. Serum Bru was taken from a PGL patient at the time of virus (LAV-1) isolation¹. Fivefold serial dilutions of sera were made and the reciprocal of the highest dilution causing >40 and >80% reduction of pseudotype PFU are tabulated.

Table 4 Neutralization of HTLV-III infectivity by selected human sera

Donor	Serum Membrane IFA titre	Surviving infectious units after antiserum treatment	
		HTLV-III	VSV(HTLV-III)
Control	0	5,000	5,000
Arb	1,250	500	500
Neb	2,500	50	500
Sel	2,500	<50	<10
Dol	1,250	5,000	5,000

Neutralization of infectivity of HTLV-III was measured by end-point titration of surviving virus after adding 1:10 dilution of antiserum to 5,000 infectious units of virus stock (cell-free culture supernatant) for 1 h at 33 °C. Duplicate flasks of HT-H9 cells¹⁴ were infected with serial 10-fold dilutions of the treated virus in the presence of 25 µg ml⁻¹ DEAE-dextran. The cells were maintained and passaged in growth medium and the percentage of cells immunofluorescent for HTLV-III membrane antigens using a known human serum assayed on days 7, 10, 13 and 18 post-infection. The cells in each flask were also tested for virus production by syncytium-inducing activity in JM cells, with similar or slightly more sensitive results than IFA. The end-point titre at 18 days (taking >10% fluorescent cells as positive) was used to calculate the surviving units of HTLV-III infecting the HT-H9 cells following antiserum treatment. The surviving plaques of VSV(HTLV-III) after similar serum treatment of 5,000 pseudotype PFU were assayed on adherent CEM cells as described in Table 1 legend.

caprine arthritis encephalitis lentivirus (CAEV)²⁷. Considerable antigenic variation is observed with EIAV and VV; antibodies elicited early in infection of individual animals have a narrow specificity of neutralizing activity, allowing non-neutralizable virus variants to emerge²³⁻²⁶, whereas later sera can neutralize a broader range of virus variants²⁵.

Antigenic variation might explain the apparently low level of neutralization of HTLV-III by human sera. However, our preliminary data show that neither early nor late serum relative to HTLV-III isolation from one patient was strongly neutralizing, and that different virus isolates supposedly made in France, the United States and the United Kingdom did not vary significantly in sensitivity to neutralization by selected sera. Preliminary experiments using human or guinea pig complement indicate that complement does not markedly enhance the inactivation of VSV(HTLV-III) pseudotypes by weakly neutralizing sera, again reminiscent of CAEV (ref 27). This raises the question of the nature of the membrane antigens recognized by human sera on the surface of HTLV-III-infected cells, which is under investigation using biochemical and immunoelectron microscope techniques.

The CD4 (T₄) cell-surface antigen is an essential and specific component of the HTLV-III receptor^{8,28}. Because neutralizing antibodies generally bind to those epitopes of retrovirus envelope glycoproteins that interact with receptors^{29,30}, the low

neutralizing response might be explained by molecular mimicry between the HTLV-III glycoprotein epitopes and an endogenous cellular antigen that normally interacts with CD4. Class II major histocompatibility complex (MHC) antigens (for example, HLA-DR) expressed on activated B cells and on antigen-presenting cells are thought to interact with CD4 antigen during the helper immune functions of CD4-positive cells^{31,32}, but we have not yet observed any anti-MHC class II antibodies that neutralize VSV(HTLV-III) pseudotypes.

Although we found no clear pattern of neutralization response among nearly 100 infected subjects, a larger proportion of PGL patients had detectable neutralizing sera than either AIDS patients or healthy seropositive individuals. The low neutralization titres may explain why patients seropositive by other techniques behave as infective carriers through sexual contact³³ or blood transmission^{34,35}. It is important to understand more about the parameters of HTLV-III neutralization for the development of effective vaccines. It remains to be seen whether HTLV-III envelope antigen manufactured by recombinant DNA techniques will yield a more powerfully protective immunogen than is apparent in humans exposed to live virus.

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HTLV-III-neutralizing antibodies in patients with AIDS and AIDS-related complex

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The isolation of the human T-cell leukaemia (lymphotropic) virus type III (HTLV-III or lymphadenopathy-associated virus) from cells of many patients with acquired immune deficiency syndrome (AIDS)^{1,2} presented the first evidence that the virus was the aetiological agent of the disease. Subsequent seroepidemiological studies have shown the presence of HTLV-III-specific antibodies in the serum of most patients with AIDS and AIDS-related complex (ARC)^{3,4}, and in the serum of many individuals at risk for AIDS⁵⁻¹⁴. Despite these extensive studies, there are no reports of protective effects of HTLV-III antibodies. In contrast, neutralizing antibodies specific for HTLV-I and -II have been identified previously¹⁵. Therefore, we investigated whether HTLV-III-exposed individuals possess antibody activities capable of inhibiting viral infection (see also refs 16, 17). Here, we report that natural antibodies capable of neutralizing HTLV-III infection of H9 cells were detected in most adult AIDS and ARC patients but in no normal healthy heterosexual controls. Geometric mean antibody titres in ARC patients were double those in AIDS patients, and were even higher in two antibody-positive healthy homosexuals. This suggests that virus neutralizing antibodies may exert an *in vivo* protective effect. The presence of these antibodies indicates an immunological response to HTLV-III which potentially may be manipulated for therapeutic advantage. The methodology used here will be useful in monitoring future vaccine approaches.

We used the H9 clone of the HT cell line¹ as a target for cell-free HTLV-III infection and initially analysed several sera for virus neutralizing activity. Infection of H9 cells was assessed by monitoring expression of HTLV-III p24 using a monoclonal antibody¹⁸ in an indirect immunofluorescence assay. Figure 1a illustrates the kinetics of infection of H9 cells with HTLV-III virus preincubated with sera positive or negative for virus neutralizing activity. By 3 days post-infection, ~80% of the H9 cells incubated with HTLV-III pretreated with control serum were infected, as indicated by their expression of HTLV-III p24. In contrast, only 10% of H9 cells expressed HTLV-III p24 at day 3 when infected with virus pretreated with serum from a patient with ARC. That inhibition of infection was mediated by a viral rather than a cellular antigen was suggested by ready infection of H9 cells with HTLV-III following pretreatment of the cells rather than the virus with the same sera (Fig. 1a). The inhibitory activity of certain sera was not simply a nonspecific effect of high serum concentrations because the activity was titratable (Fig. 1b).

We also detected neutralizing activity by other criteria. For example, two serum dilutions giving 95 and 90% neutralization

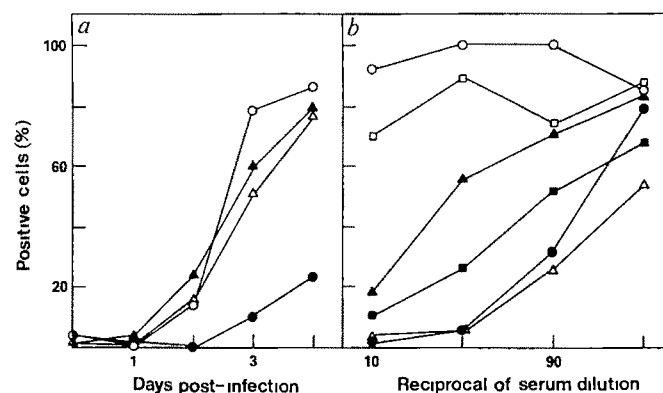


Fig. 1 *a*, Kinetics of infection of H9 cells in the presence of human serum. Sera from a normal healthy heterosexual (○) and from a patient with ARC (●). In a parallel experiment, the same sera were preincubated with H9 cells for 1 h at 4 °C. Cells were washed with PBS, incubated with the cell-free HTLV-III preparation, and cultured as described below. Results of this treatment of the H9 cells with sera are represented by the normal, healthy heterosexual serum (△) and the serum of the patient with ARC (▲). *b*, Titrations of representative human sera for virus neutralizing antibody activity. Representative titrations are shown for two sera negative for viral neutralizing antibody activity: a patient with AIDS (○) and a patient with ARC (□). Representative sera positive for virus neutralizing antibody were obtained from a paediatric AIDS case (●), a patient with ARC (■), a healthy homosexual (△) and an adult AIDS patient (▲). All values obtained were normalized to the level of infection attained in the presence of a standard antibody-negative serum.

Methods. *a*, Media containing 2.5×10^8 HTLV-III particles per ml were collected from H9/HTLV-III cells. Following removal of the cells by low-speed centrifugation, the virus-containing supernatant was centrifuged for 3 h at 32,000g in a Beckman type 35 rotor. Viral pellets were resuspended in a total volume of 2.25 ml media (RPMI 1640 containing 20% fetal calf serum and penicillin/streptomycin). Uninfected H9 cells were washed in media and incubated for 20 min at room temperature in media containing $2 \mu\text{g ml}^{-1}$ polybrene. Cells were washed and resuspended at a concentration of $4 \times 10^6 \text{ ml}^{-1}$. Sera to be tested were heat inactivated at 56 °C for 30 min and filter sterilized. For each assay, 20 μl virus suspension and 20 μl of a 1:2 dilution of serum were mixed and incubated in a microtitre plate for 1 h at 4 °C and 15 min at room temperature. H9 cells (10 μl) were added to each well and incubation continued for 1 h at 37 °C. Aliquots (15 μl) of each mixture were plated into 200 μl media in duplicate wells of another microtitre plate. Cultures were incubated at 37 °C in an incubator in which the CO_2 concentration was maintained at 5%. At the indicated time periods, cultures in individual wells were removed, washed twice with PBS and once with 1:1 PBS/water. Cells were suspended in ~30 μl of the same solution and 5-10- μl aliquots were spotted on eight-well toxoplasmosis slides. The spots were air-dried and the cells fixed for 10 min in 1:1 methanol/acetone at room temperature. Slides were stored at -20 °C before the assay. Viral infection was monitored by indirect immunofluorescence assay as described previously²⁴ on the fixed cells using ascites fluid containing monoclonal antibody BT3 (Biotech Research Laboratories) specific for HTLV-III p24 at a dilution of 1:200. Ascites fluid derived from mice inoculated with P3 $\times$ 63 cells was used as a negative control at a similar dilution. A fluorescein isothiocyanate conjugated F(ab')₂ fragment of sheep anti-mouse IgG was used as second antibody. Per cent fluorescent cells was determined following counts of at least 100 cells on each duplicate preparation. *b*, Sera were diluted in media as indicated and assayed as for *a*. Viral infection, monitored by expression of HTLV-III p24 in the indirect immunofluorescent assay, was determined at 3 days post-infection

when monitored using monoclonal anti-p24 gave 89% and 95% inhibition, respectively, when monitored using a specific monoclonal antibody to HTLV-III p15 (ref 18). Measurement of reverse transcriptase activity in proportionately increased cultures showed that the same samples neutralized viral infectivity on day 3 by 62% and 51% and on day 7 by 76% and 54%. Our system was optimized for detection of viral p24 expression in

Table 1 HTLV-III neutralizing activity is a property of IgG and is directed against a viral antigen

Serum sample	Patient diagnosis	Serum treatment	HTLV-III neutralizing antibody titre*
Experiments with purified IgG			
1	ARC	None	115
		Purification of IgG	110
2	AIDS	None	34
		Purification of IgG	40
3	ARC	None	60
		Purification of IgG	76
Absorption experiments			
4	ARC	None	135
		Absorbed with HTLV-III	<10
		Absorbed with H9 cells	120
		Absorbed with H9/HTLV-III	90
5	AIDS	None	75
		Absorbed with HTLV-III	<10
		Absorbed with H9 cells	22
		Absorbed with H9/HTLV-III	34
6	ARC	None	>270
		Absorbed with HTLV-III	50
		Absorbed with H9 cells	>270
		Absorbed with H9/HTLV-III	>270

IgG was purified from 0.5-ml aliquots of human serum by absorption to protein A-Sepharose equilibrated in phosphate-buffered saline (PBS). Following extensive washing of the columns with PBS, IgG was eluted with 0.1 M glycine-HCl, pH 2.8. The eluate was neutralized with 2 M Tris-HCl, pH 8.0, dialysed extensively against 10 mM ammonium bicarbonate and lyophilized. The purified fractions were dissolved in 0.5 ml PBS, filter sterilized and diluted in media for titration of neutralizing antibody activity as described in Fig. 1 legend. For virus absorption experiments, 62 ml cell-free virus supernatant containing 2.5×10^8 virus particles per ml were pelleted as described in Fig. 1 legend. The viral pellet was resuspended in 100 μ l of a 1:10 dilution of serum to be absorbed and incubated overnight at 4 °C. The virus was again pelleted by centrifugation and the absorbed serum was saved for titration of virus neutralizing antibody activity. Sera were similarly absorbed on pellets of washed 10^7 cells and titrated.

* Values for % HTLV-III p24-positive cells were normalized to the level of infection obtained in the presence of a standard negative serum treated similarly to the test serum. Antibody titres were then expressed as the reciprocal of the serum dilution at which virus infection was 60% of that obtained in the presence of this standard negative serum.

cells washed free of extracellular virus. The enzyme levels in culture fluids reflected not only activity in newly released virus, but also residual activity from the large virus inoculum. Ideally, precise quantitation of neutralization by enzyme assay would require a limiting inoculum and assay after 10–14 days of culture. Nevertheless, the decrease in reverse transcriptase activity obtained substantiated our hypothesis that positive sera blocked infectivity and did not simply alter p24 detection. In subsequent experiments, we assessed infectivity by p24 expression.

Serum immunoglobulins were purified on protein A-Sepharose columns and assayed for virus neutralizing activity. The serum activity capable of inhibiting viral infection resided in the IgG fractions (Table 1), indicating that the activity is immunoglobulin mediated. It is unlikely that these fractions are contaminated extensively with viral envelope capable of competing for viral receptors, or with some other non-immunoglobulin factor, but we have not eliminated these possibilities. To confirm that the inhibitory activity was directed against a viral, rather than a cellular, antigen, sera were absorbed with preparations of cell-free virus or with infected or uninfected H9 cells. Although absorption with cells had little effect, absorption with viral preparations effectively removed the inhibitory activity of positive sera (Table 1). The fact that similar titres were obtained following absorption with infected and uninfected H9 cells suggests that viral antigenic determinants involved in the neutralizing antibody response are masked or presented in a

Table 2 HTLV-III neutralizing antibody in AIDS, ARC and other at-risk patients

Serum source	HTLV-III-neutralizing antibody			Geometric mean titre $\pm$ s.e.m.
	No positive/no tested	% Positive	Range of titre	
Adult AIDS patients	21/35	60	10–520	44 $\pm$ 10
Paediatric AIDS patients	3/9	33	80–180	117 $\pm$ 28
Adult ARC patients	28/35	80	17–560	88 $\pm$ 16
Healthy homosexuals*	2/12	17	130–340	210 $\pm$ 101
Healthy heterosexuals	0/20	0	—	—
Heterosexual partners of AIDS patients†	1/3	33	78	—
Mothers of paediatric AIDS patients‡	0/2	0	—	—
Siblings of AIDS patients	1/2	50	55	—
Patients with acute mononucleosis	1/4	25	13	—
Patient with sarcoidosis	0/1	0	—	—

All sera were screened for virus neutralizing antibody at a 1:10 dilution. Those sera possessing activity were further titrated as described in Fig. 1 legend. Antibody titre is defined in Table 1 legend.

* Two of the 12 sera were also positive for HTLV-III antibodies by enzyme-linked immunosorbent assay and Western blot assay³. A third serum showed weak reactivity with p24.

† All three individuals were positive for HTLV-III antibodies.

‡ Sera from these two foster mothers were negative for HTLV-III antibodies.

|| The positive sibling was also positive for HTLV-III antibody.

different conformation on virus-infected cells compared with mature virions.

Having defined a system able to detect HTLV-III neutralizing antibodies, we analysed sera from additional patients (Table 2). A high prevalence of patients with either AIDS or ARC possess virus neutralizing antibodies, in contrast to healthy heterosexual individuals in whom no such activity was demonstrated. Although neutralizing antibody titres ranged upwards of 500 for both groups of patients, overall titres were low, but ARC patients possessed a twofold higher geometric mean antibody titre compared with that of the AIDS patients. Moreover, among healthy homosexuals at risk for AIDS, the geometric mean antibody titre (determined on only two antibody-positive individuals, and therefore not appropriate for statistical analysis) was higher than that of either of the two patient groups. Whether this trend of higher titre with less disease manifestation will be substantiated, whether it suggests a protective effect of the neutralizing antibodies, or whether it signifies a general decline in antibody titre with progression of the disease requires further investigation.

Although the number of cases examined was small, paediatric AIDS patients positive for neutralizing antibodies had elevated titres compared with those of adult AIDS patients. This difference may reflect different criteria used in diagnosing paediatric and adult AIDS cases. Among miscellaneous cases, we detected neutralizing antibodies in the serum of a heterosexual partner of an AIDS patient and in the serum of a sibling of an AIDS patient. HTLV-III neutralizing antibody activity was not detected in any healthy heterosexual individuals (Table 2). However, a very low titre was obtained in one of four serum samples from patients with acute mononucleosis. This result suggests some weak cross-reactivity with viral antigen in sera possessing high levels of heterophilic antibodies.

In other retroviral systems, the major envelope glycoprotein is the target for neutralizing antibody^{19,20}. We anticipate this will also be the case here, and studies are under way to define the antigen recognized. Whether these virus neutralizing antibodies lead to *in vivo* protection, and whether they will be relevant to future vaccine approaches, are yet to be determined. Knowledge of other retroviruses leads to the question of whether the role of HTLV-III neutralizing antibody is similar to that in the visna virus system, where infection is persistent and the resultant disease is slowly progressive. Neutralizing antibodies elicited by visna virus have a narrow range of specificity and exert a selective pressure, leading to replication of non-neutralized mutant viruses during the course of the disease²¹. This

mechanism may be relevant to AIDS because HTLV-III isolates have shown a range of genomic variability, especially in the envelope region²², and a relatedness of HTLV-III to visna virus was demonstrated recently²³. It will be necessary to obtain serial isolates and serum samples from individuals throughout the course of the disease to determine whether HTLV-III neutralizing antibodies exert selective pressure on viral mutants.

The presence of HTLV-III neutralizing antibodies in sera of individuals exposed to HTLV-III demonstrates an immunological response during the course of disease development which may be used therapeutically. Furthermore, it gives hope that appropriate vaccine approaches may be effective in preventing viral infection. Further studies will determine whether virus neutralizing antibodies in the sera of patients have prognostic value or will be indicative of appropriate treatment regimes.

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An anti-idiotypic vaccine against experimental schistosomiasis

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Schistosomiasis is a parasitic infection of man which is widespread in tropical countries, and which so far has resisted attempts at control. We have been approaching the problem from an immunological angle. We have previously reported¹ the production of a rat monoclonal IgG2a antibody against *Schistosoma mansoni* which exhibits marked cytotoxicity for schistosomula in the presence of eosinophils and a high degree of protection by passive transfer in naive rats. This antibody, IPLSm1, was shown to bind specifically to a schistosomulum membrane target antigen defined as a glycoprotein of relative molecular mass 38,000 (38K)², which is strongly immunogenic in schistosome infection of various animal species including man³. Although theoretically the 38K protein represents an excellent candidate for a potential vaccine against schistosomiasis, the glycanic nature of the epitope recognized by

IPLSm1 limits its production by DNA recombinant technology. It was, moreover, shown that, together with protective antibodies, the 38K molecule was able to induce the production of blocking IgG2c antibodies that inhibit the functional properties of IPLSm1 both *in vitro* and *in vivo*⁴. Therefore, following Jerne's network theory⁵, we considered an alternative approach, the possibility of immunization using anti-idiotypic antibodies. In the present study, rat monoclonal anti-idiotypic antibodies were produced against IPLSm1 (AB₁). Anti-idiotypic antibodies (AB₂) were selected by their capacity to inhibit the binding of radioiodinated AB₁ to its 38K target antigen. Sera from naive LOU rats immunized with a purified AB₂ preparation contained specific anti-schistosome antibodies (AB₃) which bound to 38K. AB₃ antibodies were strongly cytotoxic for schistosomula in the presence of rat eosinophils and conferred highly significant protection by passive transfer. Most importantly, rats immunized with AB₂ demonstrated marked protection (50-80%) to a challenge infection.

Monoclonal antibodies to IPLSm1 were obtained in a homologous hybridization system. Male LOU rats were injected subcutaneously with 1 mg of purified IPLSm1 plus 1 mg of glutaraldehyde-aggregated human IgG in the presence of complete Freund's adjuvant. Two weeks later, the rats received a second injection of the same preparation. The immunization was completed by two subcutaneous injections of 1 mg of

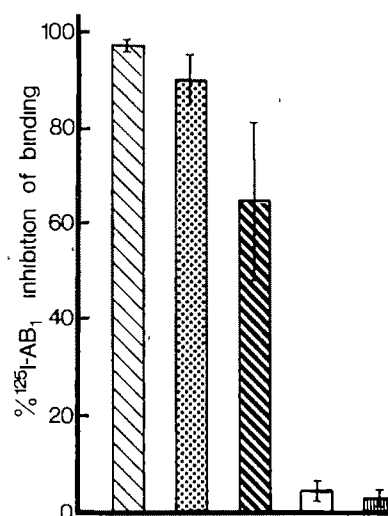


Fig. 1 Inhibition of radiolabelled AB₁ antibody (IPLSm1 IgG2a antibody) to the 38K antigen. Experiments were performed on PVC microtitre plates precoated with C₃-109 IgM antibody (an *S. mansoni* rat monoclonal antibody which recognizes the 38K molecule but does not interfere with IPLSm1 binding). Each well of the PVC plates was coated with 100 µl of a 10 µg ml⁻¹ solution of C₃-109 IgM diluted with 10 mM phosphate-buffered saline (PBS), a procedure which gives better fixation of the 38K antigen. After 2 h, the plates were washed twice in PBS buffer and saturated for 30 min with 200 µl of a 2% bovine serum albumin (BSA) solution in PBS. The plates were then washed twice in PBS-0.1% BSA. A Nonidet P-40 extract of schistosomula was added (100 µl antigen solution containing 100 µg of protein). After 2 h exposure at 37 °C, plates were washed twice in PBS-0.1% BSA buffer. For the test, 50 µl of ¹²⁵I-labelled IPLSm1 IgG2a was incubated with 50 µl of sera at a dilution of 1/25 in PBS-0.1% BSA buffer for 1 h at 37 °C and for 16 h at 4 °C and the plates were then washed three times in PBS-0.1% BSA buffer. The wells were counted in a γ-counter. The percentage of IPLSm1 inhibition binding was calculated using the following formula: $a - b/a \times 100 = \% \text{ of inhibition}$, where $a = \text{c.p.m. obtained when } ^{125}\text{I-AB}_1 \text{ was incubated with } 50 \mu\text{l of PBS-0.1\% BSA buffer}$, and $b, \text{ c.p.m. obtained when } ^{125}\text{I-AB}_1 \text{ was incubated with } 50 \mu\text{l serum from a rat bearing a subcutaneous IPLSm1 hybridoma}$ (▨), 50 µl serum from a rat immunized with AB₂ (JM8-36) (▤), 50 µl serum from a rat immunized with normal IgM (□), 50 µl serum from a 4-week-infected rat (▧) or 50 µl of normal rat serum (▩) (mean of five duplicate experiments ± s.d.).

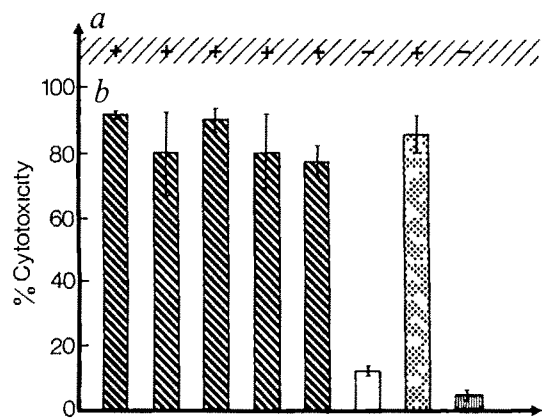


Fig. 2 Humoral response to AB₂ immunization *a*, Immuno-fluorescence tests; *b*, eosinophil-dependent cytotoxicity. Indirect immunofluorescence tests were performed¹ on deep-frozen sections of schistosomula (8 μ m) *S. mansoni* schistosomula were prepared by penetration of cercariae through abdominal mouse skin. Sera (tested at a dilution of 1/20 in 10 mM PBS) were collected from LOU/M rats immunized by JM8-36 (AB₂) antibody (▨) purified from ascitic fluid as described previously¹⁵. A 1-mg aliquot of the purified preparation in physiological saline was injected subcutaneously at 2-week intervals. Control sera were obtained from rats injected in the same conditions with the IgM fraction of normal rat serum (□). Eosinophil-dependent cytotoxicity was measured after 48 h contact of skin schistosomula, preincubated overnight with the sera at a final dilution of 1/16, with a rat eosinophil-rich population (40–60% eosinophils). The percentage cytotoxicity was compared at equivalent dilutions with control sera, normal rat serum (▨) or serum from rats infected for 4 weeks with *S. mansoni* (▩). All results correspond to a mean of two duplicate experiments $\pm$ s.d.

IPLSm1 and 1 mg of aggregated human IgG in incomplete Freund's adjuvant at 2-week intervals. The Ouchterlony test was used to select rats showing the highest responses and spleen cells from these animals were fused with cells from the IR983F rat myeloma cell line⁶ as described previously¹. The hybrid cell supernatants were screened for their ability to inhibit the binding of ¹²⁵I-labelled IPLSm1 to the 38K target antigen coated on a polyvinyl chloride (PVC) plate. From 200 hybrid cell supernatants obtained from two successive cell fusion experiments, we selected 29 supernatants inducing significant levels of inhibition (>70%).

Analysis of the immunological components of these hybrid cell supernatants by the Ouchterlony test revealed that in most cases (23/29) they contained antibodies of the IgM class. The inhibitory activity of hybrid cell supernatants on IPLSm1 binding to 38K strongly suggested that the antibodies produced were able to fix to an epitope of IPLSm1 which was close to or part of its antigen-combining site, indicating that AB₂ were paratope-induced antibodies bearing an internal image of the original epitope.

We next investigated the potential use of such antibodies to induce active immunization in naive LOU rats. Two questions in particular were studied: Is it possible to use AB₂ antibodies to induce the production of AB₃ antibodies which will reproduce the effector mechanism described previously *in vitro* both in experimental schistosomiasis⁷ and with AB₁ antibody, involving eosinophil-mediated cytotoxicity¹? Does this immunization lead to a significant protection of naive rats against a challenge infection?

To answer the first question, we followed the kinetics of the antibody response in naive LOU rats immunized with AB₂ antibodies. Successive injections of purified IgM anti-idiotype antibody (JM8-36) for 4 weeks elicited anti-schistosome antibodies (AB₃) which were detected by indirect immunofluorescence on cryopreserved *S. mansoni* schistosomulum sections. In all cases, the fluorescence reaction was observed at the surface

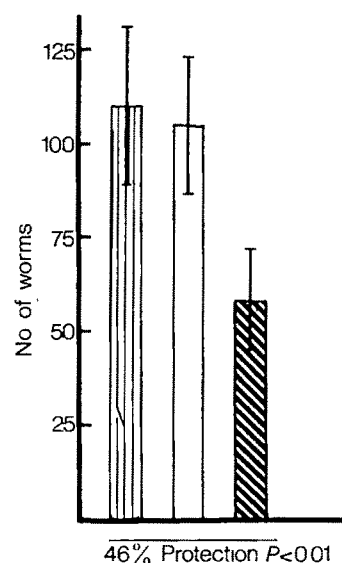


Fig. 3 Passive transfer of AB₃ antibodies. Serum (1 ml) collected from JM8-36 immunized rats (▨) or from rats immunized with normal IgM (□) was injected intravenously into LOU rats infected 4 h previously with 800 *S. mansoni* cercariae. Parasitic burdens were evaluated 3 weeks later by liver perfusion¹⁶. The number of worms obtained from these groups was compared with the parasite burden of rats injected with 1 ml of physiological saline (▨). Percentage protection was calculated by the formula $(a-b)/a \times 100$, where a = the number of worms recovered from the saline-injected control group and b = the number of worms recovered from rat injected with 1 ml of AB₃ serum.

level. These observations, suggesting a close correlation between the structure of the anti-idiotype antibody and an epitope present on the surface of *S. mansoni* schistosomula, were confirmed by competition of AB₁ by AB₃ on radiolabelled membrane preparations of schistosomula. AB₃ preparations strongly inhibited the binding of AB₁ to the 38K molecule, indicating a close specificity for the same epitope (Fig. 1).

We explored the biological activity of these AB₃ antibodies induced by anti-idiotype immunization by determining their possible participation in eosinophil-dependent cytotoxicity reactions against *S. mansoni* schistosomula. A significant level of cytotoxicity (70–90%) was observed, close to that mediated by a 4-week *S. mansoni*-infected rat serum (Fig. 2) or by the AB₁ monoclonal antibody itself¹.

The relevance of these *in vitro* findings was fully supported by the demonstration that passive transfer of AB₃ antibodies to naive rats resulted in a significant protection of these animals to a challenge infection by *S. mansoni* cercariae (Fig. 3).

Finally, we studied the direct protective effect of an anti-idiotype immunization. In two series of experiments involving 30 naive LOU rats, immunization with AB₂ resulted in 50–76% protection to a challenge infection (Fig. 4).

The work presented here clearly demonstrates that immunization with an anti-idiotype monoclonal antibody can reproduce several parameters of the acquired immunity observed in experimental rat schistosomiasis. First, immunization with AB₂ resulted in the production of specific anti-*S. mansoni* antibodies in animals which had never been exposed to the parasite antigen. The antibodies produced exhibited the same specificity for the 38K molecule as the original AB₁ monoclonal antibody used to produce the anti-idiotype antibody, suggesting that the AB₂ produced may represent an internal image of the original epitope. However, most importantly, such experiments raise the possibility of inducing a strong immunity against schistosomes, as shown by both active immunization and passive transfer experiments.

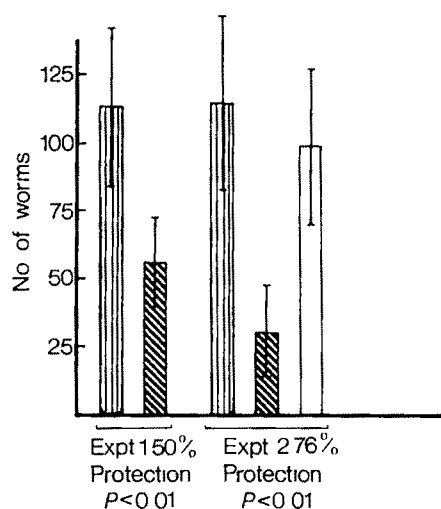


Fig. 4 Protective effect of JM8-36 immunization LOU rats immunized as described in Fig. 2 legend, giving positive immunofluorescence reactions and significant eosinophil-dependent cytotoxicity, were infected with 800 *S. mansoni* cercariae. Parasite burdens were measured 3 weeks later by liver perfusion¹⁶. The number of worms obtained from rats immunized with JM8-36 AB₂ antibodies (■) was compared with those obtained from control groups, that is LOU rats injected with physiological saline (▨) or with IgM purified from normal rat serum (□). The percentage of protection was calculated by the formula $(a-b)/a \times 100$, where a = the number of worms obtained from the saline-injected control group and b = the number of worms recovered from AB₂-immunized rats.

Thus, immunization with anti-idiotypic antibodies represents an alternative approach to immunization against pathogens. Although recent studies have produced encouraging results⁸⁻¹² concerning the potential substitution of conventional vaccines by anti-idiotypic antibodies, this strategy is only in its early stages¹³. In the context of schistosomiasis, idiotypic vaccines could be of particular use when relevant protective epitopes cannot easily be produced by the modern tools of molecular biology. Although the rat is a non-permissive host, there is now clear evidence¹⁴ that all the specific effector mechanisms of immunity described in this model also occur in human infection. The possibility of using such immunization procedures in humans remains unexplored and cannot be directly extrapolated from experimental infections. However, work in progress in our laboratory, indicating the existence of cross-reacting idiotypes in human schistosome infection, is encouraging.

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Individual-specific 'fingerprints' of human DNA

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Simple tandem-repetitive regions of DNA (or 'minisatellites') which are dispersed in the human genome frequently show substantial length polymorphism arising from unequal exchanges which alter the number of short tandem repeats in a minisatellite¹⁻⁴. We have shown previously that the repeat elements in a subset of human minisatellites share a common 10-15-base-pair (bp) 'core' sequence which might act as a recombination signal in the generation of these hypervariable regions⁵. A hybridization probe consisting of the core repeated in tandem can detect many highly polymorphic minisatellites simultaneously to provide a set of genetic markers of general use in human linkage analysis⁵. We now show that other variant (core)_n probes can detect additional sets of hypervariable minisatellites to produce somatically stable DNA 'fingerprints' which are completely specific to an individual (or to his or her identical twin) and can be applied directly to problems of human identification, including parenthood testing.

Three human minisatellites, termed 33.5, 33.6 and 33.15, each comprised of tandem repeats of various versions of the core sequence, have been cloned previously and characterized⁵ (Fig.

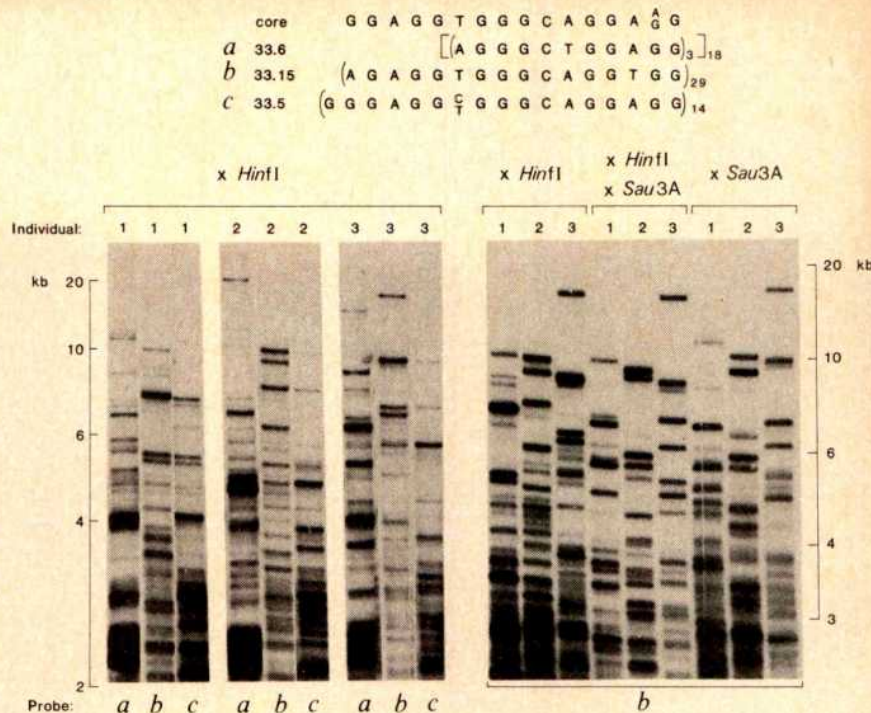
Table 1 Similarities of DNA fingerprints between random pairs of individuals

Probe	DNA fragment size (kb)	No. of fragments per individual $\pm$ s.d.	Probability x that fragment in A is present in B	Maximum mean allelic frequency/homozygosity
33.6	10-20	2.8 $\pm$ 1.0	0.11	0.06
	6-10	5.1 $\pm$ 1.3	0.18	0.09
	4-6	5.9 $\pm$ 1.6	0.28	0.14
33.15	10-20	2.9 $\pm$ 1.0	0.08	0.04
	6-10	5.1 $\pm$ 1.1	0.20	0.10
	4-6	6.7 $\pm$ 1.2	0.27	0.14

Samples (8 μ g) of blood DNA⁶ taken from a random sample of 20 unrelated British caucasians were digested with *Hinf*I and Southern blot hybridized with minisatellite probes 33.6 or 33.15 as described in Fig. 1 legend. Each DNA fingerprint (individual A) was compared with the pattern in the adjacent gel track (individual B), and the number of bands in A which were clearly absent from B, plus those which had a co-migrating counterpart of roughly similar autoradiographic intensity in B, were scored. The data shown are averages for all pairwise comparisons. A small proportion (~6%) of additional weakly hybridizing fragments in A were matched by strongly hybridizing fragments in B, and because in such cases it was not possible to decide whether the band in A was also present in B, such fragments were ignored. If co-migrating bands in A and B are always identical alleles of the same minisatellite locus, then the probability x that an allele in A is also present in B is related to the frequency q of that allele by $x = 2q - q^2$. As the allele frequency is low, then $q^2 \ll q$ and therefore the mean probability $\hat{x}$ is approximately related to the mean allele frequency $\hat{q}$ by $\hat{x} \approx 2\hat{q}$. Furthermore, assuming that there is little variance in q between alleles, then the number of alleles $n \approx 1/\hat{q}$ and the mean homozygosity is therefore approximately given by $\sum q_i^2 \approx n\hat{q}^2 = \hat{q}$. In practice, an (unknown) proportion of co-migrating bands in A and B will be derived by chance from different minisatellite loci, and thus the estimates of mean allele frequency and homozygosity are maximal and depend on the electrophoretic resolution of minisatellite fragments. Probability estimates: the mean probability that all fragments detected by probe 33.15 in individual A are also present in B is $0.08^{29} \times 0.20^{51} \times 0.27^{67} = 3 \times 10^{-11}$.

Fig. 1 Hypervariable fingerprints of human DNA. DNA samples prepared from three individual placentae (1-3) were digested with *HinfI* and/or *Sau3A* and Southern blot hybridized with ^{32} P-labelled single-stranded DNA probes prepared from M13 recombinants 33.5, 33.6 or 33.15, each of which contains a human minisatellite consisting of tandem repeats of closely related variants of the consensus sequences shown; the repeat unit in 33.6 is in turn a diverged trimer⁵. Each probe produces a different fragment pattern whose complexity is largely independent of the tetranucleotide restriction endonuclease used. Resolution of polymorphic fragments less than 4 kb long is improved in double digests with *HinfI* plus *Sau3A*, due to the elimination of background hybridization caused presumably by relatively diverged and invariant *HinfI* minisatellite fragments⁵ which have accumulated *Sau3A* cleavage sites within one or more repeat units. In double digests, the number of resolvable polymorphic fragments detected by probe 33.15 can be increased from ~15 to ~23 per individual, at the expense of losing ~20% of long single-digest minisatellite fragments which presumably contain a *Sau3A* cleavage site in most or all repeat units.

Methods. DNA was isolated from fresh human placenta as described elsewhere⁶. Samples (8 μ g) of DNA were digested with *HinfI* and/or *Sau3A*, in the presence of 4 mM spermidine trichloride to aid complete digestion, recovered after phenol extraction by ethanol precipitation, and electrophoresed through a 20-cm long 0.6% agarose gel at 30 V for ~24 h, until all DNA fragments <1.5 kb long had electrophoresed off the gel. DNA was then transferred by blotting to a Sartorius nitrocellulose filter⁷. High specific activity ($>10^9$ c.p.m. ^{32} P per μ g DNA) single-stranded M13 probes were prepared as described previously⁵. The precise probes used were: 33.5, a 220-nucleotide *HaeIII* fragment containing the minisatellite plus 60 nucleotides of flanking human DNA, subcloned into the *SmaI* site of M13mp8 (ref. 8); 33.6, a 720-nucleotide *HaeIII* fragment containing the minisatellite plus 50 nucleotides of flanking human DNA subcloned into the *SmaI* site of M13mp8; 33.15, a 592-nucleotide *PstI/AhaIII* fragment containing the minisatellite plus 128 nucleotides of flanking human DNA subcloned into M13mp19 DNA digested with *PstI* plus *SmaI*. Southern blot hybridization and washing were performed in $1\times$ SSC at 65°C as described previously⁵. Filters were autoradiographed at room temperature without an intensifier screen for 4 days.



1). Probe 33.15 has been shown to hybridize to multiple hypervariable fragments in *HinfI* digests of human DNA⁵. Probes 33.5 and 33.6 also detect a complex set of hypervariable regions in human DNA (Fig. 1). Probes 33.5 and 33.15 contain repeats of a similar version of the complete core sequence and consequently produce similar, but not identical, DNA fingerprints. In contrast, probe 33.6 is comprised of a shortened derivative of the core and hybridizes to a largely novel set of hypervariable fragments. Probe 33.15 detects ~15 resolvable hypervariable fragments per individual in the 4–20-kilobase size range, whereas probe 33.6 detects ~11 additional fragments of this size which are not detected by probe 33.15. Probe 33.5 hybridizes on average to about two further large fragments not detected by probes 33.6 or 33.15.

The DNA fingerprint pattern for the longest hypervariable fragments is largely independent of the 4-bp recognition restriction endonuclease used (Fig. 1). This strongly suggests that these large fragments are not derived from longer minisatellites, but that each contains a complete long homogeneous minisatellite devoid of restriction endonuclease cleavage sites and flanked by human DNA containing the normal high density of 4-bp cleavage sites. This is in agreement with previous data showing that most of these large minisatellite fragments are unlinked and segregate independently in pedigrees⁵.

To determine the variability of DNA fingerprints, DNA samples from a panel of 20 unrelated British caucasians were digested with *HinfI* and fingerprinted using probes 33.6 and 33.15 (Table 1). Pairwise comparisons of the DNA fingerprints showed that the minisatellite patterns were highly specific to an individual, and that few fragments are shared between two randomly selected individuals. The probability of shared bands increases for smaller minisatellite fragments, probably resulting from lower genetic variability (higher allele frequencies) of these loci⁵ combined with the fortuitous co-migration of unrelated

minisatellite fragments. From the degree of band sharing, one can obtain maximal approximate estimates of mean allele frequency and homozygosity (Table 1). For the longest minisatellite fragments in particular, the mean allele frequency is very low (<0.04) and the mean heterozygosity rises to $>96\%$. This is consistent with previous pedigree analysis which has shown that most of these large hypervariable DNA fragments are present in the heterozygous state⁵.

The data in Table 1 allow us to estimate the individual specificity of a DNA fingerprint. For probe 33.15, the probability that all 15 resolved fragments in the 4–20-kb size range in an average individual A are also present in a second unrelated individual B is 3×10^{-11} (see Table 1 for details of probability estimates); the probability that the fingerprints of A and B are identical, that is, that all fragments less than 4 kb also match and that B does not possess any additional 4–20-kb fragments, is therefore $\ll 3\times 10^{-11}$. Similarly, the probability that A and B have identical fingerprints for both probes 33.15 and 33.6 is $\ll 5\times 10^{-19}$. If individuals A and B are related, the chance of fingerprint identity is increased, to a maximum for parents/offspring and sibs. For nonconsanguineous marriages, as the heterozygosity for each fragment is very high, the probability that a hypervariable fragment in sib A is present in sib B is $\sim 1/2$, and thus the probability that all 15 resolved bands detected by probe 33.15 in sib A are present in sib B is $\sim 2^{-15} = 3\times 10^{-5}$ ($\sim 10^{-8}$ for both probes 33.15 and 33.6). These DNA fingerprints are therefore almost totally individual-specific, even within a single family (except for identical twins, see below).

The DNA fingerprints obtained using these core minisatellite probes are reproducible and are suitable for individual identification. In addition, sufficient DNA (0.5–5 μ g) can be isolated rapidly from a single drop of human blood for DNA fingerprinting. This is illustrated in Fig. 2, in which DNA fingerprints were produced from a randomized panel of individuals, including

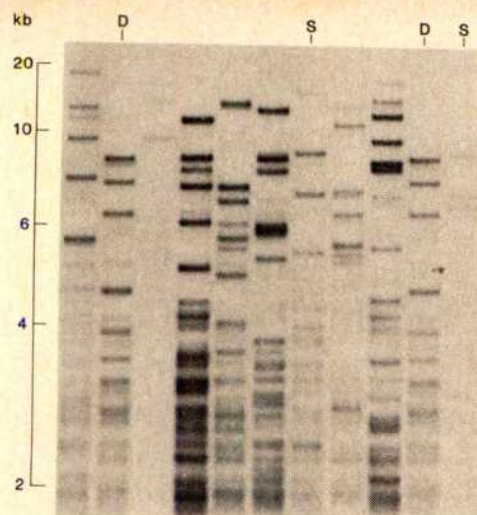


Fig. 2 Individual identification using DNA fingerprints from small samples of blood. DNA was prepared by a rapid procedure using one or two drops of blood from a panel of 11 individuals and digested with *Hinf*I; DNA fingerprints were prepared as described in Fig. 1 legend, using 33.15 as a probe. The panel consisted of nine unrelated individuals, two of whom had been previously DNA fingerprinted, plus two sisters. The autoradiograph was inspected by a colleague who was unaware of the order of samples on the autoradiograph. He correctly identified the two previously fingerprinted individuals on the basis of pattern identity, as well as identifying the two sisters, who have several bands in common (tracks S). He also correctly deduced the fact that duplicate samples had been taken from another individual (tracks D).

Methods. One or two drops (30–100 µl) of blood were collected from a fingerprick into a 1 ml 1×SSC in an Eppendorf centrifuge tube. Cells were pelleted in an Eppendorf microfuge for 1 min, haemolysed by rapid suspension in 1 ml water and immediately made isotonic by the addition of 0.25 ml 5×SSC. White blood cells, nuclei and red cell ghosts were pelleted by centrifugation for 3 min, resuspended in 0.2 ml 0.2 M Na-acetate pH 5.6 and lysed by the addition of SDS to 1%. The lysate was extracted twice with phenol/chloroform and DNA collected by two rounds of ethanol precipitation at room temperature. DNA was dissolved in 20 µl water and digested with *Hinf*I in the presence of 4 mM spermidine trichloride at 37°C for 1 h. Electrophoresis and hybridization were performed as described for Fig. 1.

two people whose DNA had been fingerprinted previously, and two sisters. The two previously characterized individuals could be readily and unambiguously identified on the basis of DNA fingerprint comparisons, as could the two sisters, who have a substantial number of minisatellite fragments in common.

We have shown previously that these hypervariable minisatellite fragments are stably inherited in a mendelian fashion, and that the mutation rate to a new length allele is low (of the order of 0.001–0.004 per locus per gamete for the longest minisatellite fragments)⁵. Several experiments show that these DNA fingerprints are also somatically stable, an essential prerequisite for identification purposes (Fig. 3). Thus, the DNA fingerprints for sperm and blood DNA are indistinguishable, as are the patterns of monozygous twins. Furthermore, the patterns appear to be stably maintained in cultured cells, as shown by comparing the DNA fingerprints of blood DNA with DNA isolated from Epstein-Barr virus-transformed lymphoblastoid cell lines derived from the same individual.

The DNA fingerprints produced by minisatellite probes 33.6 and 33.15 are therefore sufficiently stable and individual-specific for use in human identification in, for example, forensic medicine, and could be used for the routine identification and authentication of human cell lines in culture. They also provide a reliable method for paternity testing (see Fig. 3). Approximately half of the polymorphic minisatellite fragments in an offspring are derived from the father, and these paternal frag-

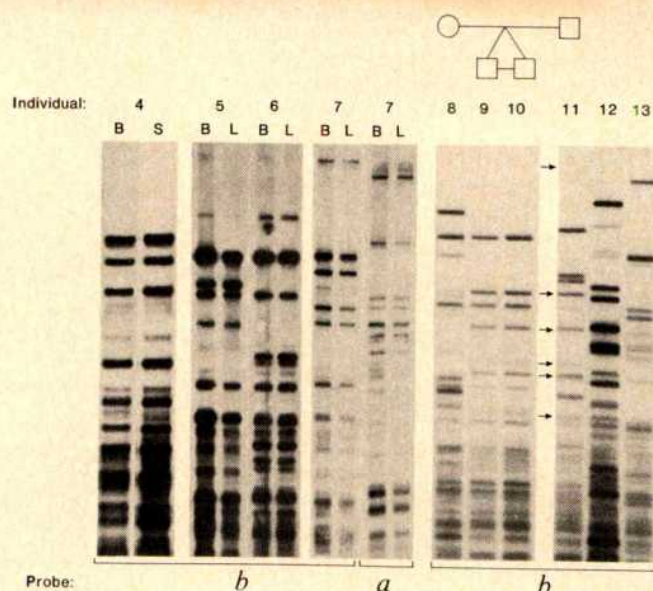


Fig. 3 Somatic stability of DNA fingerprints and their use in paternity testing. Patterns of hypervariable DNA fragments are compared between blood (B) and sperm DNA (S) of individual 4, and between blood DNA (B) and DNA isolated from transformed lymphoblastoid cell lines (L) derived from related individuals 5–7 (6 is the daughter and 7 is the maternal aunt of woman 5; the kinship is evident in the number of fragments shared by these individuals). DNA fingerprints are also shown for blood DNA from identical twins (9, 10) and are compared with their mother (8), father (11) and two unrelated men (12, 13). Resolved paternal DNA fragments in the twins (arrows) were identified by eliminating maternal bands, and are all present in the father but not in individuals 12 or 13.

Methods. Lymphoblastoid cell lines transformed by Epstein-Barr virus⁹ and stored in liquid nitrogen were re-established in liquid culture after 2 yr. These cultured lymphocytes were washed twice in normal saline; DNA from the lymphocyte pellet and from white blood cells was prepared as described elsewhere⁶. Sperm DNA was prepared similarly, except that sperm collected from semen were treated with 1 M 2-mercaptoethanol for 5 min at room temperature before lysis with SDS. DNA fingerprints were prepared as described for Fig. 1, using 5-µg samples of DNA digested with *Hinf*I and hybridized with probe 33.6 (a) or 33.15 (b).

ments can be identified by comparison of the mother's and offspring's DNA fingerprints. All of these paternal fragments must be present in the father (allowing for a possible rare new mutation). Because, in practice, probe 33.15 will detect approximately six resolved paternal fragments in the 4–20-kb range (Table 1, Fig. 3), we can use the data in Table 1 to estimate the probability that incorrect paternity will not be detected, that is, that the putative father will by chance possess all six paternal-specific DNA fragments. This probability is low ($\sim 5 \times 10^{-5}$) if the putative father is not related to the true father, but increases to $\sim 2^{-6} = \sim 0.016$ if they are closely related (brothers, father/son). If both probes 33.6 and 33.15 are used, these probabilities are reduced to $\sim 4 \times 10^{-8}$ and $\sim 8 \times 10^{-4}$, respectively, although the precise probabilities will depend on the exact resolution and complexity of the DNA fingerprint patterns obtained, and will be improved if additional paternal fragments <4 kb long can be identified (for example, by using double digests, Fig. 1). We conclude that in the vast majority of cases, the combined use of probes 33.6 and 33.15 will be sufficient to identify cases of incorrect parenthood. An interesting corollary is that these DNA fingerprints could be used with an equal level of reliability to establish true biological parentage.

This work is the subject of a UK patent application. Enquiries should be addressed to the NRDC. We thank John F. Y. Brookfield for helpful discussions, Dr R. S. Pereira for help and advice with lymphoblastoid cell lines, and Dr G. Corney and many

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DNA restriction fragments associated with α_1 -antitrypsin indicate a single origin for deficiency allele *PI Z*

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The α_1 -protease inhibitor, or α -antitrypsin (AAT), a major plasma inhibitor of leukocyte elastase and bacterial proteases, is encoded at the *PI* locus on chromosome 14 (14q24.3-q32.1)¹. A deficiency of AAT in individuals homozygous for the *PI Z* allele occurs in about 1 in 2,000-8,000 caucasians² and is associated with an increased risk of early adult onset emphysema³ and liver disease in childhood⁴. We have now used DNA polymorphisms associated with the AAT gene to investigate the origin of the *PI Z* allele. Using two genomic probes⁵ extending into the 5' and 3' flanking regions, respectively, we have identified eight polymorphic restriction sites. Extensive linkage disequilibrium occurs throughout the probed region with the *PI Z* allele, but not with normal *PI M* alleles. The *Z* allele occurs mainly with one haplotype, indicating a single, relatively recent, origin in caucasians.

Venous blood samples were collected in EDTA from 32 normal unrelated controls, 26 patients with AAT deficiency (*PI* type ZZ), and 15 pairs of parents and 16 sibs of patients with AAT deficiency. *PI* type ZZ patients included 12 adults with emphysema, 3 adults with liver disease, 9 children with liver disease and 2 healthy adults. Relatives of 16 normal controls were studied: members of 2 normal two-generation families with a total of 7 children, and 53 members of a three-generation family of a *PI* ZZ proband which included 12 unrelated spouses. The genetic type of AAT (*PI* type) was determined by isoelectric focusing of plasma in acrylamide gels, pH 3.5-6 (ref. 6). Blot hybridization of leukocyte DNA digested with specific enzymes

is described in Fig. 2 legend. The 5' probe (4.6 kilobases, kb) and 3' probe (6.5 kb) have been described elsewhere⁷ (Fig. 1), both having been subcloned from phage clone α AT35⁵.

Of 10 restriction enzymes tested with the 5' 4.6-kb probe, 3 showed restriction site polymorphisms; these were *Sst*I, *Msp*I and *Ava*II. For each enzyme, one or both of two possible restriction fragments were observed. When a particular restriction site was present (designated +), a shorter DNA fragment was observed than when the restriction site was absent (designated -). Family studies indicated that the pair of DNA fragments for each enzyme were alleles. Among 32 unrelated normal controls, the allele frequencies were as shown in Table 1. The 4.6-kb genomic probe has been completely sequenced⁸, so the positions of the restriction sites were identified. Fragments of the expected sizes were observed. The distances between restriction sites were 2 kb between *Sst*I and *Msp*I sites, and 0.2 kb between *Msp*I and *Ava*II sites.

A χ^2 analysis of 2x2 tables, or exact calculation of probabilities where required⁹, was used to examine the data for possible association between a specific allele at one site and a specific allele at each of the other two sites. No significant association between alleles was observed, indicating free recombination between the three restriction sites, with no evidence

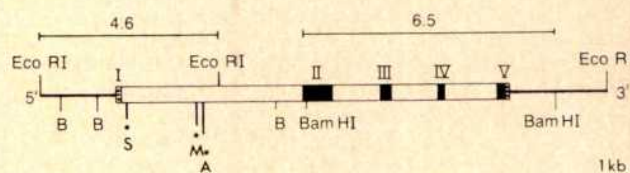


Fig. 1 The α_1 -antitrypsin gene and adjacent flanking regions, described previously^{4,17}. Solid areas are coding regions, dots indicate untranslated regions, open areas are introns. Restriction sites for the enzymes *Eco*RI and *Bam*HI (B) are indicated. *, Polymorphic restriction sites for *Sst*I (S), *Msp*I (M) and *Ava*II (A). The two genomic probes used in this study are shown above the gene.

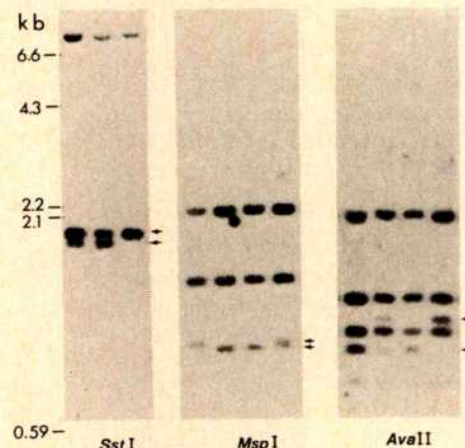


Fig. 2 Patterns of DNA fragments are shown for the three enzymes *Sst*I, *Msp*I and *Ava*II, using the 4.6-kb 5' probe. DNA extracted from leukocytes was completely digested with specific restriction enzymes according to conditions recommended by the manufacturer. Fragments were separated by size in 0.8% agarose gels. DNA fragments were transferred to Biodyne membranes (Pall). Probes were labelled with ³²P using a nick-translation kit (Amersham). After prehybridization, ³²P-labelled probe (3.0x10⁷ counts per filter) was hybridized overnight. Filters were rinsed twice in 2xSSC (SSC: 1.5x10⁻⁴ M NaCl, 1.5x10⁻⁵ M Na-citrate). Membranes were washed in 2xSSC at 60 °C for 45 min, then twice in 0.1% SDS in 0.1xSSC at 60 °C for 45 min, rinsed briefly in SSC, blotted, then exposed to X-ray film for 2-3 days. Fragment sizes in kb are indicated on the left. Anode is at the bottom. Arrows mark the polymorphic fragments. From left to right, lanes show the following allele designations: +-, ++, --, --, ++, ++, +-; ++, +-, ++, --.

Table 1 Allele frequencies for 5' DNA polymorphisms (4.6-kb probe)

Restriction enzyme	Alleles (kb)	Allele frequency +	Allele frequency -	PIC*
<i>Sst</i> I	1.8, 1.9	0.649	0.351	0.35
<i>Msp</i> I	0.95, 0.98	0.506	0.494	0.38
<i>Ava</i> II	0.9, 1.1	0.636	0.364	0.36

* Polymorphism information content, calculated according to the formula of Botstein *et al.*²¹, expressing the extent of variability at each locus.

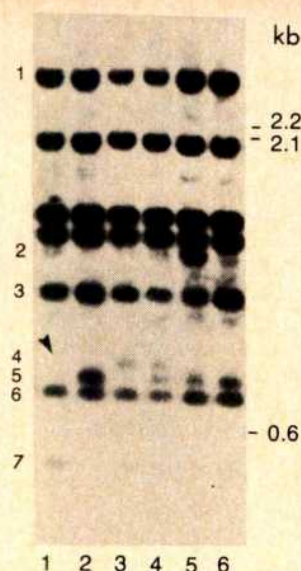


Fig. 3 DNA fragments observed using restriction enzyme *Ava*II and the 6.5-kb probe. Sizes of fragments are indicated in kb, anode is at the bottom. The seven polymorphic fragments are numbered on the left. There are also three strong bands present between polymorphic bands 1 and 2 which appear to be constant in all samples observed to date. The *Ava*II DNA phenotypes observed in each lane are as follows: (1) 11336677; (2) 11336655; (3) 10334677; (4) 10334657; (5) 11236657; (6) 11336657. The arrow in lane 1 shows the unique region of the pattern associated with PI type ZZ.

of linkage disequilibrium between them in normal individuals, despite the close proximity of sites. The specific combination of the three restriction sites (DNA haplotype) was determined by segregation within families. Forty-seven non-Z haplotypes (M of various subtypes and S) could be determined from 16 normal controls and from 15 parents of PI ZZ children using only the non-Z haplotype. The specific haplotypes found with each PI type are shown in Table 2. The expected haplotypes for all non-Z PI types were calculated on the assumption that each restriction site polymorphism segregates independently, so that the probability of a particular haplotype occurring is the product of each of the component allele frequencies. There is no significant difference between observed and expected frequencies ($\chi^2 = 10.7$; d.f. = 7) for the data available (Table 2), although the $-++$ and $-+-$ categories may be decreased in larger series. Again, the conclusion is that there has been considerable recombination between the three restriction sites, with no linkage disequilibrium for non-Z haplotypes. The DNA haplotypes found in combination with specific PI types are shown in Table 2. Further data will be required to determine whether there is linkage disequilibrium between each specific PI type and specific haplotypes, although our data suggest there is disequilibrium for PI variants M3 and S.

In contrast to the results for the normal non-Z PI types, among the 26 unrelated individuals with AAT deficiency (PI type ZZ), 25 were homozygous for the haplotype *Sst*I $-$, *Msp*I $+$, *Ava*II $+$. The haplotype $-++$ therefore occurred with 51 of 52 PI Z alleles. One PI Z allele was associated with the 5' DNA haplotype $+++$, indicating a crossover event between the *Sst*I and *Msp*I sites, or a mutation at the *Sst*I site. There is therefore a very high degree of linkage disequilibrium for the 5' restriction sites of PI Z alleles. This specific haplotype ($-++$) is found in association with 8 of 28 PI M1 alleles and 1 of 13 M2 alleles, suggesting that the PI Z allele has been derived from the PI M1 allele. The amino-acid sequence of the Z AAT product differs in one position from that of M, subtype undefined: at position 342 in exon V, glutamine in M is changed to lysine in Z^{10,11}.

Further polymorphisms were found using the 3' 6.5-kb probe and the restriction enzyme *Ava*II. The pattern of DNA fragments

included at least seven polymorphic fragments and three constant fragments, ranging in size from 0.48 to 2.7 kb (Fig. 3). Because sequencing is incomplete for the region of the 6.5-kb probe, not all specific sites have been identified, so fragments have been numbered. The polymorphic band 1 (2.7 kb) was either present or absent, indicated as alternate alleles 0 or 1, with frequencies of 0.36 and 0.64, respectively. The fragment labelled 2 (1.15 kb) (Fig. 3, lane 5) represents a rare allele, frequency 0.02. Bands 4 (0.72 kb) and 6 (0.64 kb) are alleles, frequencies 0.30 and 0.70, respectively. At least one constant fragment is also present in position 6. Alleles represented by bands 5 (0.68 kb) and 7 (0.48 kb) have frequencies of 0.07 and 0.30, respectively. Fragment 7 transfers inefficiently and can usually be seen clearly only when present in the homozygous state. The proposed interpretation of alleles is supported by family studies.

The phenotype observed in lane 1 of Fig. 3, with both bands 4 and 5 absent, was not observed in 32 normal control individuals. However, this band pattern was observed in all of 26 individuals of PI type ZZ. The particular combination of *Ava*II DNA fragments can be stated as a haplotype. PI type ZZ individuals are therefore homozygous for the haplotype 1367. Of the 47 non-Z haplotypes determined by family studies, none has the 1367 haplotype. The most common *Ava*II haplotype is 1365, found with 27 of 47 non-Z alleles (11/28 M1, 10/13 M2, 4/4 M3 and 2/2 S). This haplotype would require only one mutation to produce an *Ava*II restriction site to convert fragment 5 to fragment 7. About 75% of the region of the probe has been sequenced⁸ and fragments of the sizes of polymorphic bands 2, 3, 4, 5, 6 and 7 are located within the AAT gene. Two additional restriction site polymorphisms, one within the gene and one in the 3' flanking region, were observed using the 3' probe and the restriction enzyme *Taq*I (data not shown). The polymorphic *Taq*I sites are identical for 51 of 52 Z haplotypes; the exception involves a *Taq*I site 3' to the AAT gene.

The finding of a single unique DNA haplotype for most (96%) PI Z alleles is exceptional for diseases studied to date. The first association of a DNA restriction fragment polymorphism with a disease was described for sickle-cell anaemia¹². Multiple restriction sites associated with the sickle-cell haemoglobin gene indicate three independent origins in Africa¹³. For thalassaemia, a specific restriction fragment haplotype is generally associated with a specific mutant gene and haplotypes tend to be characteristic for defined ethnic groups¹⁴. In contrast, for AAT, the mutant gene is almost always associated with a single haplotype, indicating a single origin for the PI Z allele. While most patients in the present study are of British origin, others are Ukrainian, German, Dutch and French. Many different population groups have now been surveyed², and have shown that the PI Z allele is limited to caucasians and is not found in oriental or black population groups, except where it can be explained by racial admixture. Also, there is a suggestion of a decreasing gene frequency from north to south in European populations. The

Table 2 DNA restriction fragment haplotypes (5' 4.6-kb probe) associated with specific PI types

Haplotype			Total non-Z		PI type*				
<i>Sst</i> I	<i>Msp</i> I	<i>Ava</i> II	Observed	Expected	M1	M2	M3	S	Z
-	-	-	6	3.0		6			
-	-	+	1	5.2	1				
-	+	+	9	5.3	8	1			51
-	+	-	1	3.0	1				
+	-	-	5	5.5	1	4			
+	-	+	11	9.6	9			2	
+	+	-	5	5.6	5				
+	+	+	9	9.8	3	2	4		1
Totals			47	47	28	13	4	2	52

* Controls were selected before PI typing; frequencies of PI types therefore reflect population frequencies²².

PI Z allele has certainly arisen since the divergence of the races, and probably much more recently. The number of generations which have elapsed since the origin of the *PI Z* mutation can be estimated, assuming that meiotic crossing-over is a random event (which is not strictly true) and that on average there is roughly one meiotic crossover per 10^8 base pairs per generation¹⁵. As the crossover frequency is 1 in 52, and the estimated distance between the most 5' restriction site (*Sst*I) and the most 3' *Ava*II polymorphic site is approximately 9×10^3 base pairs, it can be calculated that 216 generations, or 6,480 yr, would be required to generate 1.9% crossovers. Because no crossovers have occurred in the 7-kb distance between the 5' *Msp*I site and the most 3' *Ava*II site, the number of generations may be considerably less. The lack of linkage disequilibrium between the three 5' polymorphic restriction sites suggests an ancient origin for the polymorphisms, or a region of high recombination. Differential rates of recombination have been shown within the β -globin¹⁶ and immunoglobulin¹⁷ clusters. After the single

origin in a northern caucasian race, selective forces may have increased the frequency of the *PI Z* allele (see ref. 2 for a review). Such selective forces may be direct; AAT may be directly involved in the fertilization process and the decreased concentration in *PI Z* heterozygotes could enhance fertility^{18,19}.

In addition to providing information on the origin of the *PI Z* allele, the studies reported here show that various clinical manifestations of the disease are associated with only one mutant allele. Furthermore, the unique *Ava*II haplotype provides a basis for prenatal diagnosis²⁰.

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Spontaneous excision of a large composite transposable element of *Drosophila melanogaster*

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The *TE1* family of transposable elements (TEs) of *Drosophila* consists of unusually large transposons, cytologically visible in larval polytene chromosomes as one or more bands¹⁻³. They are composite elements, as their termini consist of foldback (FB) sequences which are themselves transposable⁴⁻⁶. The location of FB elements at the termini of transposable elements suggests that these sequences have a direct role in the genetic instability of TEs. To investigate the structural and phenotypic consequence of TE excision, we have cloned genomic DNA required for the expression of the *no-ocelli* (*noc*) gene of *Drosophila*; this gene has been mutated by the insertion of *TE146*, a member of the *TE1* family carrying six polytene chromosome bands including functional copies of the *white* (*w*⁺) and *rough* (*rst*⁺) genes. As reported here, our experiments indicate that the spontaneous excision of *TE146*, which results in the loss of the *w*⁺ and *rst*⁺ markers, can occur either as a single-step event or following a partial internal deletion. In either case, the end product is an imprecise excision in which a residual portion of the element, varying in size from 3 to 10 kilobases (kb), is left at the insertion site. These residual sequences share homology with the FB family. Furthermore, despite their imprecise nature, all these spontaneous excisions restore a wild-type *noc*⁺ phenotype.

The first transposable element identified arose from the spontaneous transposition of two adjacent genes, *w*⁺ and *rst*⁺, from

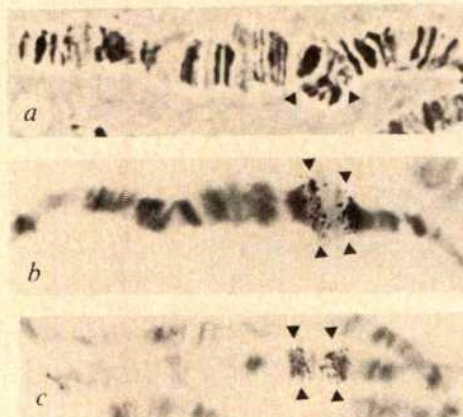


Fig. 1 *a*, Polytene chromosomes of a *TE146/+* heterozygote, showing the six bands of the TE inserted near bands 35B1.2. Arrowheads indicate the cytological limits of *TE146*. *b*, Polytene chromosomes of homozygous *TE146* hybridized with a nick-translated probe prepared from a clone (M365; ref. 6) including sequences from the *w*⁺ gene. There are two bands of homology to the probe within the TE and one at 3C1, the normal locus of *w*⁺. Arrowheads indicate sites of hybridization within *TE146*. *c*, Polytene chromosomes of homozygous *TE146* hybridized with a nick-translated probe prepared from a clone (FB8) including sequences of the repetitive FB element^{4,5}. Note that there are at least two discrete sites of hybridization within the TE (arrows).

their normal location on the X chromosome to chromosome arm 2R. The element (*TE1*) resulting from this original transposition is mobile and its derivatives have been mapped to over 150 cytological locations throughout the *Drosophila* genome².

Transposable elements can induce mutations in genes that lie at, or near, their insertion sites; for some, but not all, of these TE-induced mutations, the subsequent loss of the element leads to a restoration of the wild-type phenotype². To understand this observation, and to clarify the role of FB sequences in TE instability, we examined the structural consequences of TE loss. For this purpose, *TE146*, a member of the *TE1* family which

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Fig. 2 a, Polytene chromosomes of TE146/CyO hybridized with a genomic clone, λ ob 9.04, that crosses the site of insertion of the TE. The single site of hybridization to the wild-type homologue (open arrowhead) is split into two by the insertion of the TE (solid arrowheads). Cy indicates the left breakpoint of the *In(2L)Cy* component of CyO. **b**, Restriction enzyme site map of a 25-kb genomic region of *Iss2* (the wild-type strain carrying the chromosome into which the TE had integrated to form TE146) which spans the TE insertion site. Recombinant clones containing the two 12-kb genomic *EcoRI* fragments which comprise this region were isolated from an EMBL-4 library constructed from *Iss2* DNA by virtue of homology with the insert of λ ob 9.04 (see text). The *EcoRI* fragments were subsequently subcloned into pUC9. The extents of λ ob 9.04 and of subclones which were used as probes are shown as solid lines above the restriction map. The TE is represented by the large triangle. Restriction enzyme sites internal to the element were determined from Southern transfer experiments. The site of insertion lies within the 700-bp *HindIII/SalI* fragment and is less than 50 bp from the *SalI* site, the *SstI* site located just within the proximal terminus of the TE being less than 50 bp from this *SalI* site. For this reason, the 700-bp *HindIII/SalI* fragment (probe 2) will only detect fragments spanning the distal junction of the element. R, *EcoRI*; Xh, *XhoI*; Ss, *SstI*; B, *BamHI*; Sa, *SalI*; H, *HindIII*; Xb, *XbaI*. **c**, *Iss2* and TE146 genomic DNA was digested with *HindIII/SalI*, fractionated on a 0.8% agarose gel and transferred to nitrocellulose membrane. The filter was hybridized to probe 4, ^{32} P-labelled by nick-translation, then washed and autoradiographed. Lane 1, *Iss2*; lane 2, TE146. Label was removed from the filter with two washes in 0.1 M NaOH at room temperature then it was re-hybridized to probe 2, washed and autoradiographed. Lane 3, *Iss2*; lane 4, TE146.

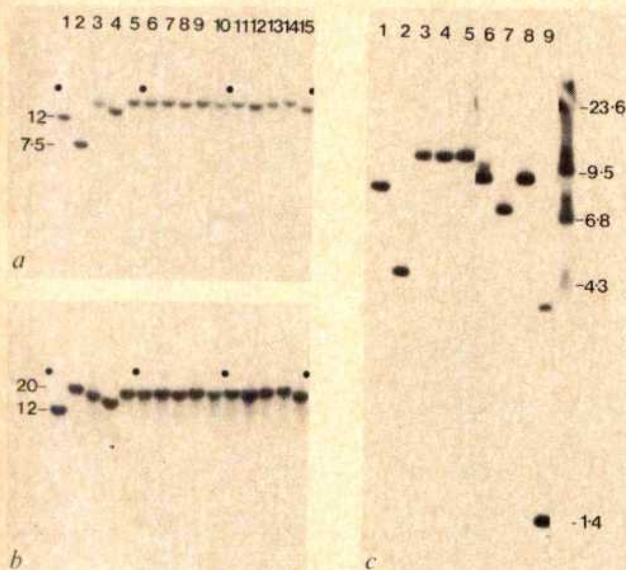
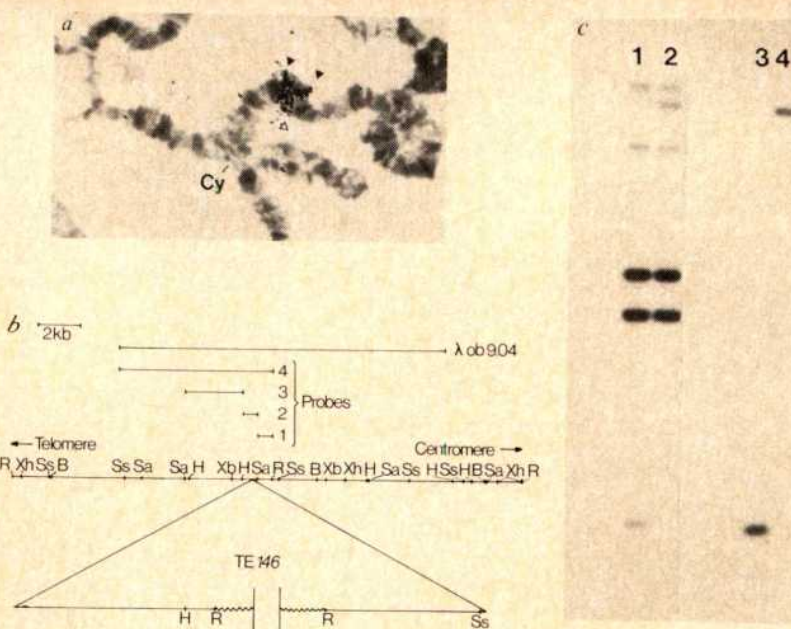


Fig. 3 Restriction enzyme-digested DNA was fractionated on 0.8% (a, b) and 0.6% (c) agarose gels and transferred to nitrocellulose membrane. The filters were hybridized to the probes indicated below, then washed and autoradiographed. Where the same filter was re-used for different probes, label hybridized to the filter was removed by washing with 0.1 M NaOH before hybridization with the subsequent probe. **a**, All samples were digested with *EcoRI*. Lane 1, *Iss2*; lane 2, TE146; lanes 3–15, different strains carrying independent spontaneous excision chromosomes (hybridized with probe 1). **b**, The same filter used for **a** was hybridized to probe 2; an identical result was seen when probe 3 was used. **c**, All samples were digested with *EcoRI/HindIII*. Lanes 1–8, different strains carrying independent spontaneous excision chromosomes; lane 9, *Iss2*. Markers used are *HindIII*-digested λ DNA. Identical results were obtained with either probe 1 or 2. The faint 4-kb band in lane 9 results from partial digestion with *HindIII*. The lanes in **c** and **a** which correspond to the same excision derivatives are as follows: (tracks in **c** are indicated first) 1 = 2, 2 = 4, 3 = 5, 4 = 9, 5 = 8, 6 = 12, 7 = 15; track 8 in **c** is not represented in **a**.

contains two copies of w^+ , was chosen because chromosomes from which this element had been spontaneously lost were available, as was a large region flanking its insertion site. In larval salivary gland chromosomes, this element contains six chromosomal bands, equivalent to several hundreds of kilobases of DNA (see Fig. 1a). Like other members of the TE family, TE146 shares homology with the cloned *w* and *FB* sequences (Fig. 1b, c). Both copies of the *w* genes carried by TE146 are functional, and spontaneous excision events have previously been isolated by selecting for the absence of w^+ (ref. 7). The site of insertion is associated with *noc*, a gene that is two complementation groups distal to the alcohol dehydrogenase (*Adh*) gene on chromosome arm 2L⁸. Flies carrying homozygous deletions of the *noc* gene are viable, but lack ocelli and associated bristles. TE146 abolishes *noc*⁺ function, so it is associated with a strong recessive *noc* mutation.

The insertion site was sufficiently close to *Adh* to be cloned by chromosomal walking⁹. Using the cloned *Adh* gene as a starting point¹⁰, overlapping DNA fragments were isolated from a wild-type Canton-S library¹¹ until the insertion site was reached. That DNA spanning the insertion site of TE146 had been obtained was shown by *in situ* hybridization to larval salivary gland chromosomes. The phage clone λ ob 9.04, containing DNA located roughly 100 kb distal to *Adh*, hybridizes with both sides of the element (Fig. 2a); it therefore contains DNA sequences spanning the insertion site. Using as a probe the *Drosophila* DNA contained within λ ob 9.04, we isolated a 25-kb segment of DNA from a phage bank constructed from the genomic DNA of the strain of flies carrying the chromosome into which the TE had integrated to form TE146. The restriction map of this DNA, and Southern transfer experiments which localize the insertion site to a 700-base pair (bp) *SalI/HindIII* fragment, are shown in Fig. 2b, c.

We have analysed eight independent spontaneous excision chromosomes derived from TE146 which, in addition to having lost the w^+ markers, are cytologically wild type at the insertion site. Southern filters of gel-fractionated DNA digests from these strains were prepared, and the filters were hybridized to two different probes, one with sequences adjacent and distal to, the other with sequences adjacent and proximal to, the insertion

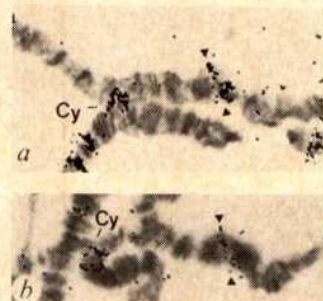


Fig. 4 *In situ* hybridization of the FB8 probe to two different chromosomes that have spontaneously lost TE146 (see Fig. 1c). The arrow indicates FB homology at bands 35B1.2. Cy indicates the left break of *In(2L)Cy*: a, TE146(Z)SW22/CyO; b, TE146(Z)SW13/CyO.

site (see Fig. 2 legend). Figure 3a, b shows the resulting autoradiograms. In TE146 the distal probe hybridizes to a 20-kb *Eco*RI band which spans the distal junction of the element and adjacent chromosomal DNA, whereas the proximal probe hybridizes to a 7.5-kb band corresponding to the proximal junction. By contrast, each of the lanes containing spontaneous excision chromosome samples contains a single band which appears to be homologous to both probes; this is clearly one band which hybridizes to both probes, and not two different bands of the same size, because consistent results were obtained when this experiment was repeated after the DNA had been cut with different restriction enzymes (data not shown). As homology to both the 700-bp proximal and distal probes is observed, these excision events have not deleted more than 700 bp of chromosomal DNA sequences immediately flanking either side of the insertion site. The simplest interpretation of the data is that these events resulted from the deletion of most, but not all, of the several hundred kilobases of DNA sequence associated with the element. The amount of TE DNA remaining in these chromosomes can be determined from the increased sizes of the wild-type 1.4-kb *Eco*RI/*Hind*III fragment spanning the transposable element insertion site. The fragment varies between 3 and 10 kb in the excision chromosomes examined (Fig. 3c). *In situ* hybridization experiments indicate that the remaining DNA shares homology with cloned FB sequences (see Fig. 4).

FB elements are implicated in mediating these excision events because of their location at the termini of elements belonging to the TE1 family³. The terminal structure of FB elements, combining short tandem repeats within large inverted repeats, could promote homologous pairing, in and out of register, between the inverted repeats⁴. Where two FB elements flank an intermediate sequence, as in members of the TE1 family, pairing and recombination between two repeats (one from each FB element) in the same orientation will result in the loss of the intermediate sequence and the retention of a variable portion of the FB sequences. The loss of DNA flanked by FB elements has been shown previously for two *w* mutations, white-crimson (*w^c*) and Dominant zeste-like (*w^{DZL}*)^{12,13}. There are, however, two differences between the loss of TE146 from *noc* and the losses of the FB-associated insertions from *w*: (1) losses of TE146 are selected on the basis of a function carried by the element itself, rather than a function which is affected by the position of the element; (2) the DNA separating the FB elements is <14 kb for *w^c* and *w^{DZL}*, whereas we estimate a minimum of 200 kb for TE146.

While the events described above, in which essentially all of the TE is lost in a single generation, occur relatively infrequently ($\sim 5 \times 10^{-5}$) (ref. 7), partial deletions of the element, which result in the loss of only one copy of the *w⁺* gene, can occur at much higher frequencies ($\sim 5 \times 10^{-4}$). These partially deleted chromosomes retain several polytene bands associated with the transposable element, and may result from exchange between a

terminal FB element and one internal to the TE, separating its two *w⁺* genes. These partial deletions do not revert *noc*, but subsequent loss of the remaining bands can restore a wild-type *noc⁺* phenotype (D.G. *et al.*, unpublished data). Such losses of TE146 in two stages, via the partial deletion intermediate, result in chromosomes which are indistinguishable from the one-step excision events already described; they retain <10 kb of FB-homologous sequences at the insertion site. The length of the insertion varies among different independently derived losses (data not shown).

Paro *et al.*¹⁴ have suggested that the presence of FB elements at the termini of the TE1 family may be the cause of their genetic instability. The results presented here indicate that the excision of the six intervening chromosomal bands of TE146 from their site of insertion could result from recombination between the terminal FB elements of the transposable element. The imprecise nature of these excisions provides an explanation for the lack of correlation between excision and reversion for most of the mutations induced by this family of elements^{1,2,15}. In the case of TE146, however, all the imprecise excision events, regardless of their mode of origin, restore a wild-type *noc⁺* phenotype. It would be difficult to rationalize this observation if TE146 had disrupted *noc* function by inserting into a coding portion of the gene. A possible explanation of the reversions is that the insertion of several hundred kilobases of DNA in close proximity to *noc* can disrupt its expression by virtue of a position effect. For example, the TE could alter the local chromatin structure and thereby affect *noc* expression. In this case, excision of the element, despite being imprecise, could restore normal expression by removing most of the material responsible for the structural alteration. A similar position effect observed for the *w^{DZL}* mutation is also removed by the deletion of DNA sequences between two FB elements.

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Identification of myosin heavy chain in *Saccharomyces cerevisiae*

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Motility in biological systems is expressed in a variety of ways, such as cytoplasmic streaming, cell shaping, nuclear migration and muscle contraction. These functions are thought to be mediated by structural proteins, for example, myosin, actin and tubulin (see ref. 1 for review). The involvement of myosin in muscle contraction is well documented and this protein is implicated in generating the cleavage forces during cytokinesis in some non-muscle cells².

Here, we report the isolation of a protein similar to myosin as judged by its biochemical and immunological properties, from the yeast *Saccharomyces cerevisiae*. Parts of the protein have been conserved through evolution at the protein and DNA sequence levels. The presence of this protein in the region bordering mother cell and bud, as revealed by immunofluorescence, suggests that it is involved in cell division.

In an attempt to isolate novobiocin binding proteins from yeast, we applied total cell extract to a novobiocin-Sepharose column³ and eluted the bound proteins with ATP, urea or increasing salt concentrations. Under these experimental conditions, several proteins were eluted in 1 M KCl or 5 M urea (data not shown). Among these were two proteins of relative molecular mass (M_r) $200,000 \pm 5\%$ present in both fractions. The high M_r of these proteins, combined with the fact that novobiocin inhibits the ATPase activity of bacterial topoisomerase type II through competition with ATP³, led us to examine whether one of these proteins could be a myosin heavy chain, which is known to contain an ATPase activity.

We first used polyclonal⁴ and monoclonal⁵ antibodies raised against nematode muscle myosin, in the Western blot technique⁶. The polyclonal IgG fraction recognizes the smaller of the two high M_r proteins (Fig. 1a, lanes 1, 3). Two monoclonal antibodies (out of a set of 22) raised against nematode myosin interact with the same yeast protein (Fig. 1a, lane 4), supporting this result. One of these antibodies (28.2) interacts with a moderately conserved region of the S2 proteolytic fragment of the nematode myosin heavy-chain isoform encoded by the *unc-54* gene⁵.

Several biochemical criteria have been proposed by Pollard⁷ and others to ensure a positive identification of myosin. The most important of these are its ability to form a complex with actin and its ATPase activity, sometimes stimulated by actin but always dependent on either Mg^{2+} or Ca^{2+} ions. The Ca^{2+} -dependent activity is especially striking, as other known cytoplasmic ATPases are almost exclusively Mg^{2+} -dependent and are usually inhibited by Ca^{2+} . Therefore, this activity is generally used to follow the purification of myosin from crude cell extracts. Table 1 shows that the ATPase activity of the 1 M KCl fraction eluted from the novobiocin-Sepharose column is actin activated (using purified rabbit actin) and is either Mg^{2+} or Ca^{2+} dependent.

To discover whether the putative myosin can form a complex with actin, we isolated actomyosin complexes under conditions similar to those described by Pollard⁷. Yeast spheroplasts were lysed in 10 mM Tris-HCl pH 7.9, 1 mM dithiothreitol; sodium pyrophosphate and sucrose were then added to final concentrations of 50 mM and 0.34 M, respectively, to ensure complete myosin solubilization. After a high-speed clearing spin (30,000 r.p.m.), the supernatant was dialysed against 10 mM sodium citrate, pH 6.5, to precipitate the actomyosin complex. The complex was collected by centrifugation at 30,000 r.p.m.; it is composed primarily of a M_r $200,000 \pm 5\%$ protein (Fig. 1b)

Table 1 ATPase activity of two crude myosin preparations from *S. cerevisiae*

	Novobiocin-Sepharose 1 M KCl fraction ($\mu\text{mol min}^{-1} \text{mg}^{-1}$)	Actomyosin complex ($\mu\text{mol min}^{-1} \text{mg}^{-1}$)
No additions	≥ 5	≥ 5
Mg^{2+}	150	220
Mg^{2+} /actin	250	220
Ca^{2+}	100	90
K^+ /EDTA	20	10

Assays of ATPase activity were performed in 25- μl aliquots containing 25 mM Tris HCl pH 7.5, 1 mM ATP and one of the following: (1) 5 mM $MgCl_2$; (2) 5 mM $MgCl_2$, 1 mg ml^{-1} rabbit actin; (3) 5 mM $CaCl_2$; (4) 20 mM KCl, 1 mM EDTA. The 1 M KCl fraction had been dialysed to remove the KCl. Inorganic phosphate was measured according to Lanzetta *et al.*¹³. The actin preparation did not contain any detectable ATPase activity.

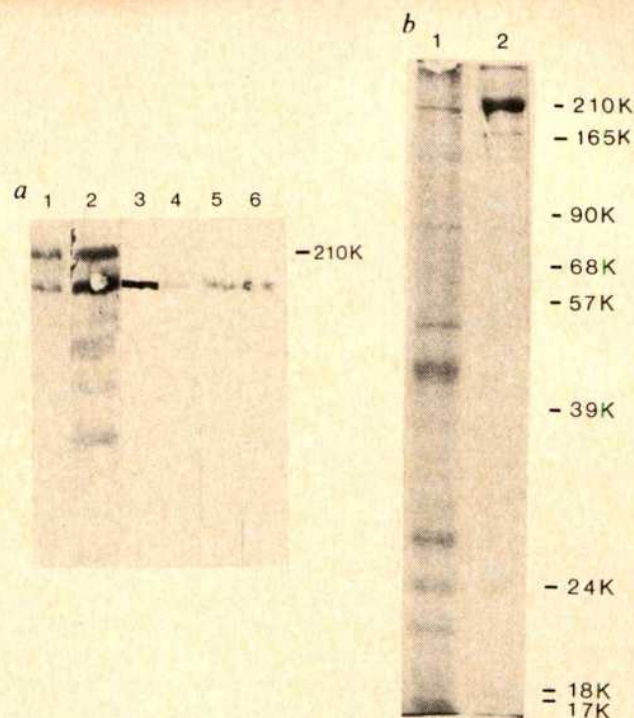


Fig. 1 SDS-polyacrylamide gel electrophoresis and Western blot analysis of crude myosin preparations. *a*, Yeast spheroplasts, prepared using $50 \mu\text{g ml}^{-1}$ zymolyase in 1.2 M sorbitol were lysed in 50 mM Tris-HCl pH 7.9, 1 mM dithiothreitol, 50 mM KCl, 1 mM p-enylmethylsulphonyl fluoride. The resulting solution was subjected to centrifugation at 30,000 r.p.m. for 1 h. The supernatant was loaded onto a novobiocin-Sepharose column, which was then washed with 0.1 M KCl followed by 1 M KCl. Actomyosin complexes were precipitated using a modification of the method described by Pollard⁷. The 1 M KCl fraction (lanes 1-4) and actomyosin complex (lanes 5, 6) were analysed on a 5% SDS-polyacrylamide gel¹⁴ and either (lanes 1 and 5) stained with Coomassie brilliant blue or (lanes 2-4, 6) electroblotted to nitrocellulose⁶. The filter in lane 2 was stained for protein using India ink¹⁵. The filter in lane 3 was incubated with rabbit serum containing polyclonal antibodies against nematode myosin, followed by goat anti-rabbit IgG-peroxidase, while the filters in lanes 4 and 6 were incubated with a monoclonal antibody 28.2 against nematode myosin, followed by goat anti-mouse IgG-peroxidase. M_r marker (210 K) is rabbit myosin heavy chain. *b*, The actomyosin complex was analysed by SDS-polyacrylamide gel electrophoresis (12.5%). Lane 1, yeast actomyosin complex; lane 2, rabbit myosin heavy and light chains. M_r markers (from top to bottom) are rabbit myosin, *Escherichia coli* RNA polymerase, bovine serum albumin and pyruvate kinase.

and actin (as determined with yeast anti-actin IgG in an enzyme-linked immunosorbent assay and on a Western blot, data not shown). Western blot analysis showed that the monoclonal antibodies against nematode myosin reacted with the M_r 200,000 protein (Fig. 1a lanes 5, 6), strongly suggesting the presence of the myosin heavy chain in the complex. The ATPase activity of this complex is again either Mg^{2+} or Ca^{2+} -dependent (Table 1) but is not stimulated by actin, presumably because the latter is already present in sufficient quantities in the complex. In general, myosin is a hexameric molecule composed of two heavy chains and four light chains, each of $M_r \sim 20,000$. Thus far, we have not directly identified myosin light-chain molecules in yeast, although minor proteins of appropriate M_r are co-precipitated in the actomyosin complex (Fig. 1b).

The significance of the immunological data has been further investigated by Southern blot hybridization. A *Bam*HI fragment encoding the ATP-binding region of a nematode myosin heavy chain was hybridized to total genomic yeast DNA. This fragment spans a region that is conserved within the nematode myosin

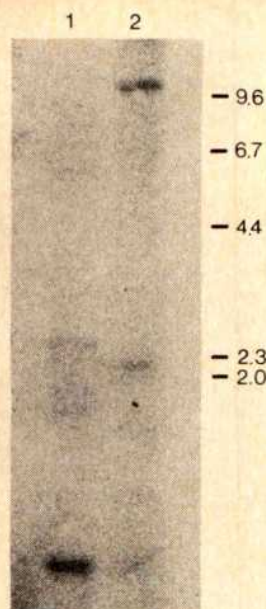


Fig. 2 Southern blot analysis¹⁶ of *S. cerevisiae* genomic DNA with a nematode myosin DNA probe. Genomic DNA from A364A was digested with *EcoRI* (lane 1) or *BamHI* (lane 2), run on a 0.7% agarose gel and blotted to nitrocellulose. The filter was incubated with a nick-translated *BamHI* fragment, containing the region of a nematode myosin heavy-chain gene encoding the ATP-binding site⁸, in $2\times$ SSC (0.3 M NaCl, 30 mM sodium citrate) at 60 °C. Size markers are *HindIII* fragments of λ phage DNA.

heavy-chain gene family. Protein sequences in this fragment contain high homology to the ATP-binding region of myosin heavy chains from various organisms⁸. Figure 2 demonstrates that this probe hybridizes to a 1.2-kilobase (kb) *EcoRI* fragment and to a *BamHI* fragment of ~10 kb. A second DNA probe, carrying a less well-conserved sequence from the S2 region of the nematode *unc-54* myosin heavy-chain gene did not hybridize to yeast DNA (data not shown).

The detection of myosin heavy chain in yeast raises some interesting questions regarding its physiological roles in this and similar organisms. The possible role of myosin in non-muscle cells, as well as that of actin in yeast, have been suggested by their detection using *in situ* immunofluorescent techniques^{2,9,10}. Adopting a similar approach, we used either the nematode anti-myosin IgG or mouse serum containing anti-yeast myosin IgG (our unpublished results). Fixed cells were treated with anti-myosin IgG followed by a secondary IgG coupled to fluorescein isothiocyanate (FITC). Most of the fluorescence was localized at the junction between mother cells and buds (Fig. 3a). This fluorescent band becomes visible as soon as buds can be detected and disappears at the completion of cell division.

These data suggest that myosin is involved in the mechanism of budding, perhaps analogous to its assumed function during cytokinesis in higher eukaryotic cells², which do not have rigid cell walls and in which myosin is associated with the contractile rings of dividing cells. Actin has also been visualized at this neck region in *S. cerevisiae* by the immunofluorescent technique⁹. In this case, however, staining appeared immediately before bud emergence and at the final stage of cytokinesis, but was not detected during bud emergence, presumably because of structural changes in this region during budding. The pattern of staining observed with anti-myosin antibodies coincides with the appearance of 10-nm filaments in this region¹¹. It has been suggested by Kilmartin and Adams⁹ that the actin structures are not associated with the 10-nm filaments in yeast. However, other results (D. Botstein, personal communication) clearly demonstrate that actin is present at the neck region throughout the budding process. We are currently analysing yeast cell-division cycle (*cdc*) mutants that lack the 10-nm filaments at the restrictive temperature¹² to determine whether these filaments contain myosin.

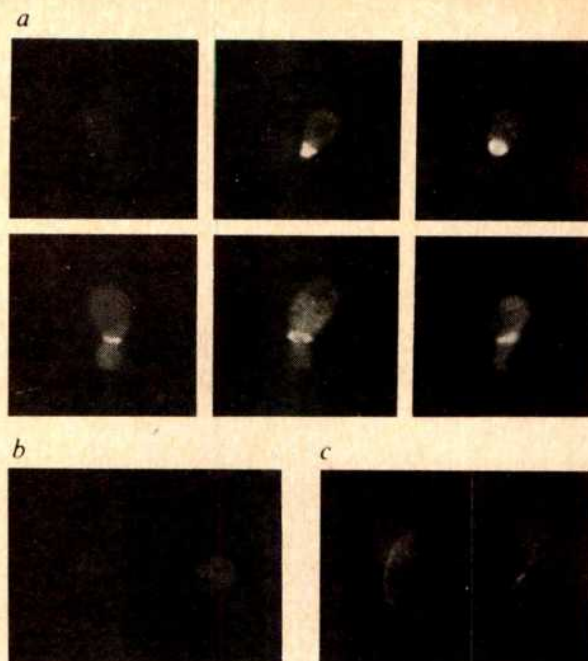


Fig. 3 Fluorescence micrograph of yeast cells treated with anti-nematode myosin antibodies and anti-yeast tubulin antibodies. Immunofluorescence was performed using a modification of the method described by Kilmartin and Adams⁹. Cells were fixed with 3% formaldehyde and spheroplasts were prepared using 100 μ g ml⁻¹ zymolyase. Spheroplasts were incubated with: *a*, serum containing polyclonal anti-nematode myosin antibodies, followed by goat anti-rabbit IgG-FITC; *b*, non-immune serum followed by goat anti-rabbit IgG-FITC; *c*, monoclonal anti-yeast tubulin antibody⁹ followed by anti-rat IgG-FITC.

It has been widely speculated that, as well as its involvement in cytokinesis, myosin is a component of the cellular cytoskeleton. It has been proposed recently that actin participates in the transport and secretion of newly synthesized components for the budding cell wall in yeast^{9,10}. We hope that further analysis of mutants defective in the myosin gene will elucidate its interaction with actin and its function in the cell *in vivo*.

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India-Eurasia collision chronology

Patriat and Achache's analysis¹ of the convergence between India and Eurasia led them to conclude that India collided with Eurasia around anomaly 22 time (~50 Myr), confirming an earlier interpretation based on geological relationships in northwestern India that collision was pre-Middle Eocene², probably around anomaly 23 time³ (~52 Myr). Geological evidence from the Lhasa region^{4,5} also suggests that obduction of the suture-zone rocks onto the leading edge of India occurred there in the pre-Eocene interval, and that any significant shortening in the Lhasa Block was complete before the end of the Palaeocene⁶.

These observations pose a problem for Patriat and Achache's model¹ because in their Figs 1b and 4, 50 Myr ago southern Tibet was located some 7° north of the presently exposed northern boundary of India. Patriat and Achache¹ suggest that the 700 km of continental crust necessary to fill this gap was consumed first by subduction of ~400 km of Indian continent beneath southern Tibet from 50 to 44 Myr, and then by continued continental underthrusting and internal deformation to account for another 300 km of north-south shortening before 37 Myr. There are at least three problems with this model.

(1) Deformation in the supposed continental subduction and underthrust zone (Indus-Tsangpo suture) should be of Eocene age, rather than late Cretaceous or early Palaeocene as shown by field relationships^{4,5}.

(2) The now-vanished continental crust would have lain between the Tethyan Himalaya and southern Tibet, in which case the Tethyan Himalaya would have lain in the interior of the Indian continental block before collision, and not at its leading edge as is widely accepted^{2,6,7}.

(3) Southern Tibet would have had a much thicker than normal continental crust from mid-Eocene to present, in which case isostatic rebound would have led to a Palaeogene rise of the Tibetan plateau, for which there is no geological evidence.

These problems disappear if the pre-collisional shape of India is extended northwards to take account of its original size in Gondwanaland^{8,9}. A pre-collisional Greater India with its northern edge extending as far north of the exposed Indian Shield as the Kun Lun is today would not only have collided with southern Tibet by the postulated 50-Myr collision time, but could later have underthrust its broken-off leading edge, the Tethyan Himalaya, to produce the 70-km continental crust beneath Tibet. The underthrusting could have been as a series

of intracontinental slices¹⁰ rather than a single continental underthrust^{2,3}.

Recognition of the Tethyan Himalaya as the leading edge of Greater India obviates the necessity to postulate 700 km of Eocene continental subduction and underthrusting beneath southern Tibet, and raises the possibility that the threefold sequence of events suggested by Patriat and Achache occurred in almost exactly reverse order. Initial shortening within Eurasia is taken up by block rotation and lateral extrusion along sinistral strike-slip faults¹¹, then followed by crustal thickening through internal deformation and the beginning of continental underthrusting, and finally, when the continental underthrust zone is sufficiently well established, large-scale continental subduction occurs^{2,9}.

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ACHACHE AND PATRIAT REPLY—Powell *et al.* estimate the pre-collisional shape of India after 'its original size in Gondwanaland'. This coincides with the present position of the Kun Lun belt. We think this method is highly questionable and thus, such an estimate must be considered as speculative.

The problems allegedly raised by our model call for the following comments: (1) The Cretaceous to Palaeocene F₁ deformation observed in the Yarlung Zangbo suture zone is related to northward subduction of oceanic crust^{1,2}. It is followed by a larger-scale although less intense younger F₂ deformation which can be associated with crustal subduction and shortening^{1,2}.

(2) The observation of Tethyan sediments in no way contradicts the model of subduction of 400 km of continental crust and only requires a 500-km-wide continental shelf north of India, before the collision. Whether this can still be considered as the so-called leading edge of India is merely a matter of vocabulary.

(3) The age of rise of the Tibetan plateau is still an open question. In particular, there is no valid evidence against a Palaeogene rebound.

We therefore do not see any real objection to our model in the comment by Powell *et al.* Furthermore, several field observations seem to contradict the reversed sequence of events suggested by these authors. First, a recent palaeomagnetic study³ of Tethyan sediments in Tibet has shown the amount of shortening in the MCT to be 400±400 km, thus much less than required by the proposed model. Second, structural and geochronological data indicate that significant underthrusting occurred in the Indian continent (Kangmar Thrust, Main Central Thrust, Main Boundary Thrust) between 45 and 35 Myr ago (see ref. 3). And third, the magnetic anomaly pattern in the South China Sea indicates that the motion along the Red River fault (and thus the extrusion of Indochina⁴) did not start until anomaly 13 time (36 Myr), that is, 15 Myr after the beginning of the collision (see ref. 5).

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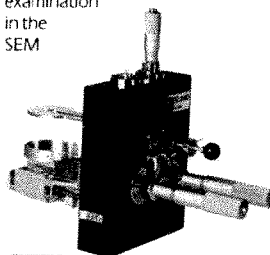
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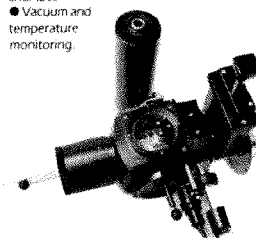
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Image analysis takes off

from Geoff Jenkinson

Recent software developments are taking image analysis into new and uncharted areas of science.

THE basis of the image analysis method is well-known and it is not intended to describe this here. However, the architecture of the system is relevant in defining the capabilities of an image analysis system. A modern instrument, for example the Quantimet 920, will consist of a number of integrated components as shown in Fig. 1.

The video processor takes in the analogue video signal from the scanning source, either a TV-type scanner or a scanning electron microscope. The scan standards are chosen to optimize the resolution and signal potential of the source and give over 630,000 image points with up to 256 grey levels. A built-in shading corrector compensates for scanner and optical grey level variation. The resulting signal is converted to digital form and can be stored as a full grey-level image.

The 2-D array processor performs real-time image transformations to remove errors caused by optical and electronic effects which limit the rise-time of the video signal, to enhance fine detail, for example in grain boundaries, and to remove specimen-dependent shading. The image processor performs other digital signal processing tasks and also extracts a wide range of field and feature data parameters from the image. The image store can store multiple grey images at a variety of resolutions and binary images. These images may be roamed and zoomed and transferred to and from disk.

Software

Users of image analysis systems have an enormous variety of requirements. The software has to be structured to meet all these needs and can be broadly considered

Fig. 2 Image analysers can now be packaged together with a scanning electron microscope, as on this Quantiscan 922 from Cambridge Instruments.



as falling into three main types:

Turnkey application program. A program or set of programs is provided which requires only that the operator follows a sequence of prompts which appear on the display. This will be normally used for routine applications which only require the operator to make minor adjustments to settings of detection or for interactive image editing for example.

Interactive routine building. To create a new application solution the user is provided with a special ready-to-use interactive software package known as QUIPS. This allows the various image analysis facilities to be called up and the effect of selecting various combinations made immediately visible. Test results may be examined to decide if it is worthwhile proceeding to a more detailed analysis. In operation the primary image analysis functions are displayed on the control monitor and these are selected by means of a pointing finger which is manipulated via a digitizing tablet.

Software extensions. The potential of the system is not limited to the standard functions as described above. A PASCAL software package allows complete freedom of access to all data and images in the system, so that new functions and instructions can be defined and incorporated into the standard routines.

Application examples

Two applications have been chosen which illustrate the range of techniques required of image analysis systems. These are all in routine use on standard production instruments.

Receptor binding autoradiographs. In quantitative autoradiography in neurobiology and pharmacological research, image analysis provides facilities for region selection of the autoradiography, measuring radiation levels and colour-coding images. Image analysis can signifi-

cantly ease the interpretation of results and by means of its speed, make possible experiments which could not previously be contemplated.

In the study of receptor binding the general approach can be illustrated by quantifying the binding of a drug to rat forebrain sections. Adjacent sections are incubated both with and without a drug which displaces a radioligand from the binding site or sites which are being investigated. Thin sections of brain tissue are used to expose a tritium-sensitive film which produces an autoradiograph in which the radiation levels give a corresponding density on the film. Standards of radio-labelled tissue are also exposed on the film. A calibration curve is produced which converts the grey-level on the film to the quantity of the bound drug.

The adjacent sections produce images which represent total binding and non-specific binding of the drug. A single section of total binding is imaged at low magnification onto the TV camera of the image analyser and stored in its image memory. Corrections of non-uniform illumination and conversion to radiation via the calibration curve are made before storage. The image of the nonspecifically bound section is superimposed with the outline of the previous section and similarly stored. Subtraction of the nonspecific bound image from the total bound image now gives an image which represents the specifically bound component. The percent specific binding can now be determined by dividing the specific bound image by the totally bound image. Regions of interest may be outlined by means of the digitizing tablet and the mol per mg protein within the region determined.

An alternative method of measuring the radiation values is to use a higher magnification and dark-field illumination on a light microscope which allows the individual grains in the photographic emul-

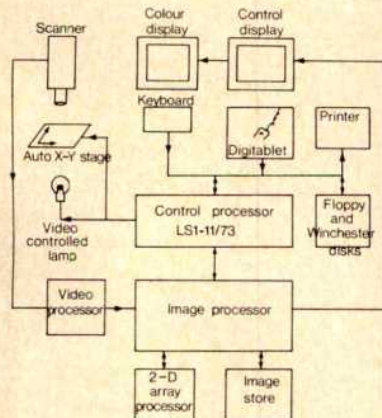


Fig. 1 Block diagram of a modern image analysis system.

sion to be seen and hence counted. This method is used when smaller regions need to be identified or when the density of the autoradiograph is low.

Combined scanning electron microscopy and image analysis. The potential of combined scanning electron microscopes and image analysis systems has been acknowledged for some years. The SEM gives superior resolution and excellent contrast on many specimens which cannot be analysed by means of a light microscope. A variety of detectors is available for the SEM which form images corresponding to different properties of the specimen and which are suitable for image analysis. Up to now however it has been difficult to capitalize on this potential due to the relative complexities involved in connecting the two instruments together. It is now possible to fully integrate the capabilities of the electron optics and image analysers, as is done in the Quantiscan 922 system shown in Fig. 2. This analyser has a resolution better than 4nm in secondary electron imaging mode when fitted with a directly heated LaB₆ emitter producing an overall magnification range of 4× to 300,000×.

The SEM image is transferred directly to the image store in the image analysis system from where it can be continuously displayed on the screen of the analyser. Transfer rate is selectable from 0.3s up to 200s, and calibration of the measurements is made automatic by monitoring the SEM operating conditions.

When used in conjunction with an energy dispersive X-ray system the analytical potential is further extended. The X-ray information indicates the presence of particular elements in a specimen, and the importance of this is that it may be combined with the topographic and structural information from the image analysis to give a complete interpretation of the specimen.

Other applications

Image analysers can be used for many other applications and have found ready acceptance in quality control in fibre industries where they are used to measure the characteristics of synthetic fibres, in electronics to map the dislocation density in semiconductor wafers and to monitor oil contamination in hydraulic systems. In the field of metallurgy, where image analysers have been in use for more than two decades, their utility is being extended by the introduction of automatic charting.

There is also increasing demand for fast simple image analysers capable of routine particle sizing and counting and these are incorporating many of the features of their larger relatives. This will continue the downwards cost trend which will make image analysis available to an even greater range of users in the future. □

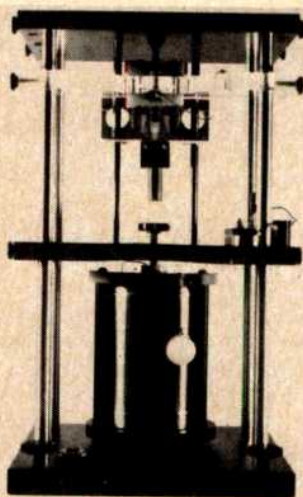
Geoff Jenkinson is at Cambridge Instruments Ltd, Rustat Road, Cambridge CB1 3QH, UK.

Image analysis and microscopy

A sampling of products from a simple magnifier to the latest in image analysis.

- Capable of analysing images from microscopes, video-tape recorders, drum scanners, photometers and photographs, Reichert-Jung's Polyscan offers maximum flexibility of image format without compromising access to the powerful software library. The system is programmable using either direct routines from an extensive menu or custom modifications entered via the image processing library. Polyscan will run automatically and uses a high resolution, low geometric distortion camera, capable of better than 1,000 lines, and computer-controlled beam blanking for image integration. Pseudocolour processing is supplied as standard and real colour is available as an option. Two CRTs are included in the system: a 12-inch black and white VDU for displaying user dialogue and programming; a 13-inch high resolution colour monitor for the image being analysed.

Reader Service No. 100.



Gentleman Jim for rapid freezing.

- A novel method for quick freezing tissue samples for electron microscopy is adopted in a new system from Pelco. 'Gentleman Jim' is an instrument which permits instant freezing of biological samples by dropping the selected tissue onto a polished surface of pure copper at liquid nitrogen temperature. Gentleman Jim does not permit bounce during contact as a special hydraulic system forces the sample tissue to maintain contact during the crucial initial moments of quick freezing. Excellent results have been obtained for the first 12 to 15 micrometres of tissue depth. Helium is unnecessary since liquid nitrogen produces satisfactory results. After freezing, dehydration by freeze substitution may be carried out.

Reader Service No. 101.

These notes are based on information provided by the manufacturers. To obtain further details about these products use the reader service card bound inside the journal.

- The DM54K image analyser from Digital Measurement Associates Ltd. provides a high speed low cost approach to obtaining quantitative measurements from subjective images. The system comprises a television camera interfaced to an Apple IIe computer via a custom interface enabling video images to be digitized in real time, approximately one fiftieth of a second. The interface expands the Apple working memory to 128K and also provides eighty-column text facility. It is supported by a suite of dedicated software providing a full range of image analysis features. The TV camera may be attached to a standard viewing system to image macro objects, photographic prints and so on. Alternatively a standard range of adaptors is available for light microscopes. TV images are digitized in real time to provide a detected matrix of 280 × 192 pixels. A possible 256 grey levels are available in either 'blacker than', 'whiter than' or 'grey slice' modes together with a programmable shading correction system to adjust for uneven background illumination. Area, end count, full feature count and intercept data are generated as a basis for a full stereological analysis.

Reader Service No. 102.

- The new 40-10 model image analyser from AMS (Analytical Measuring Systems) is available with a dedicated biological software package for applications such as colony counting, bacteria counting, breakpoint MIC determination, inhibition zone measurement, viral plaque counting and spiral plate counting. The VIDS II semi-automatic image analyser is a graphics tablet based system and is used in conjunction with an Apple computer and Epson printer. The scanner provided with the system can be mounted on any type of microscope or used with the AMS macro stand for macro-samples. With this system any sample that can be imaged with the camera can be "drawn round" and measured. Typical applications include analysis of histological sections, SEM and TEM micrograph measurements and particle size analysis.

Reader Service No. 103.

The AMS 40-10 image analyser.



IMMUNOLABELLING FOR ELECTRON MICROSCOPY

edited by J. M. Polak and I. M. Varndell

Immunolabelling for Electron Microscopy is a compilation of invited chapters written by highly experienced and active field workers, which documents established principles and current trends in all aspects of electron microscopical immunocytochemistry. The chapters cover the history of the technological achievements, all currently practised electron immunocytochemical techniques, often with innovative modifications, and the application of these procedures for the morphological identification of antigens from tulip plant viruses to man.

This volume is primarily directed towards the electron microscopist who has questions for which immunocytochemistry could provide the answer. The intended audience is, however, very much wider and taxonomists to cell biologists, endocrinologists to diagnostic pathologists, both in routine and research laboratories, will profit from the book's coverage of this rapidly emerging and important field.

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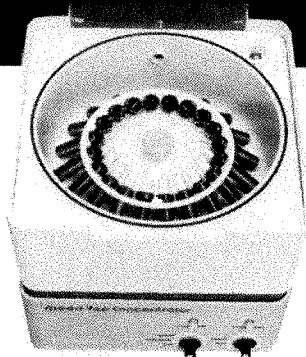
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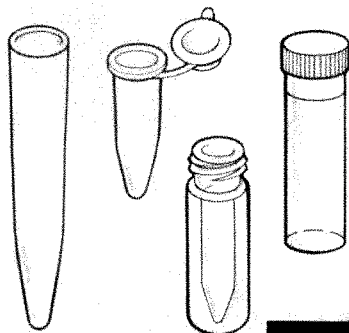
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A Nimbus microcomputer forms the basis for the latest image analysis package from Digithurst.

- As an alternative to supplying image analysis equipment for connection to existing microcomputers, Digithurst has introduced an image analysis system based around the Research Machine's Nimbus. The latest version of the Digithurst Micro-Sight is incorporated in the system which enables images to be captured at up to 512×512 resolution with 265 grey levels. Using standard graphics the image can be displayed at 256×256 with 16 grey levels on the Nimbus screen. The system uses an 80186 CPU running at 8MHz 320K of memory and twin floppy disk drives; hard disk drive versions are available together with networking options. The memory can be expanded up to 1 megabyte. Images captured by the system can be dumped to a printer or stored onto disk, they are also available to graphics packages which can manipulate and edit the displayed pictures in applications such as art, design, advertising, display and computer aided learning.

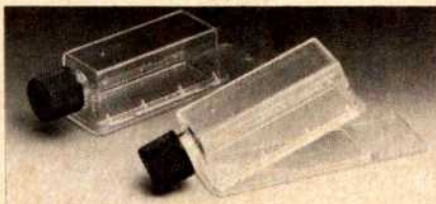
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- Raymond A. Lamb's Labstack filing system offers a wide range of stackable steel cabinets for storage of microscope slides, either vertically or horizontally in slotted racks. Transparency cabinets are also available as well as paraffin block filing cabinets with divided drawers to hold either System I embedding rings or System II embedding cassettes. These cabinets are in two sizes, either standard for floor stacking or half-depth for bench-top use. The drawers are provided with stops to prevent accidental removal although they can be removed and relocated. Accessories include spring spacers for initial filing before the mountant has hardened for contact storage, adaptors for holding larger slides in 26 mm wide drawers, different coloured location slips, transparency filing cards, slide wallets and dividers to file 76×26 mm slides in 52 mm wide drawers. System I embedding rings and System II embedding cassettes are included in the range together with cardboard boxes in varying sizes for unmounted blocks.

Reader Service No. 105.

- Philips Analytical has introduced a new Edax EDSCAN software package which provides a means of acquiring and presenting spatially related chemical information from a sample using a scanning electron microscope with attached Edax 9100 energy dispersive analyser. The package uses computer controlled microscope beam positioning to probe the specimen and collect from it X-ray and electron back-scattered signals quickly and efficiently. Collection beam geometries range from single spots to digitized line scans and area scans. Data is stored on disk and can, if required, be easily manipulated using simple arithmetic processing before presentation on one of a variety of available output devices. The Fortran-based EDSCAN software package is divided into two distinct functions: XSCN, devoted to X-ray signal collection, and ESCN for microscope signal acquisition.

Reader Service No. 106.



Nunc's flask-come-slide.

- The Nunc SlideFlask is a combination of a cell culture flask and a microscopy slide. The bottom for the flask has exactly the same dimensions as a standard microscopy slide, giving a culture area of 9 cm^2 . It is manufactured from Nunclon Delta material for good cell adherence and optical clarity. The two flask parts are welded together, but slide and flask can be separated at any step in the preparation procedure after the cells have been cultured. The Nunc SlideFlask is specially designed for culture and preparation of cells and subsequent microscopic examination of the specimen under high power magnification (for applications such as chromosome examination, single cell autoradiography and single cell immunofluorescence).

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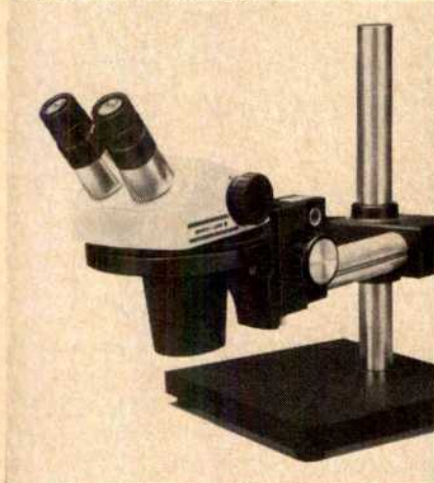
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Polaron's new sputterer.

• Although sputter coaters are now in routine use in the scanning electron microscopy laboratory for the coating of biological and other types of insulating materials with gold or some other noble metal, it has hitherto proved impossible to sputter materials such as aluminium or carbon which are necessary as a conducting coating for microprobe techniques. But the Polaron E5800 plasma enhanced a.c. sputtering system successfully deposits aluminium and other refractory materials. The plasma in the vicinity of the sputtering target is enhanced by an intense magnetic field; sputtering voltage is typically 300V, 150mA. Sputtering rates for aluminium are typically 60nm per second. If a gold target is used for routine SEM coating, then coating times can be as short as 15–20 seconds, avoiding heating problems on the sample surface.

Reader Service No. 108.



StereoZoom 6 from Bausch & Lomb.

• StereoZoom 6, the latest microscope from Bausch & Lomb, has improved optics designed to match the colour correction response of the human eye. It provides clear, crisp images, true colour rendition and sharp contrast. Standard features include a one-to-six zoom system with an 0.67 to four basic magnification range, sharp resolving power at higher magnification, a wide field of view and exceptional depth of field at lower magnification (0.55 mm at 1.0 $\times$). The instrument's InstaZoom makes it possible to run through the entire zoom range with less than one-third of a revolution of the control, and its narrow, 56mm objective end allows it to be used in tight spots.

Reader Service No. 109.

• LKB-Produkter AB of Sweden has introduced a new high-precision glass-knifemaker (LKB 2178 KnifeMaker II) which allows the user to prepare 45° knives in either of two ways: in the traditional way or by applying the new 'balanced break' concept. No special skills are required to produce knives for semithin light microscopy sectioning or for cutting ultrathin sections for ultramicrotomy and cryo-ultramicrotomy. In the balanced break technique a standard 400-mm long strip of glass in any of three thicknesses (6.4, 8 or 10mm) is continuously broken up into equal halves to produce a full set of flat-sided 25 $\times$ 25 mm squares. The KnifeMaker II introduces two new scoring positions, to give four scoring choices. By enabling optimal corner-to-corner breaks, it simplifies the production of 45° angled cryo-knives. The holder also has adjustable click-steps, useful when preparing 40, 50, 55 and 60° knives.

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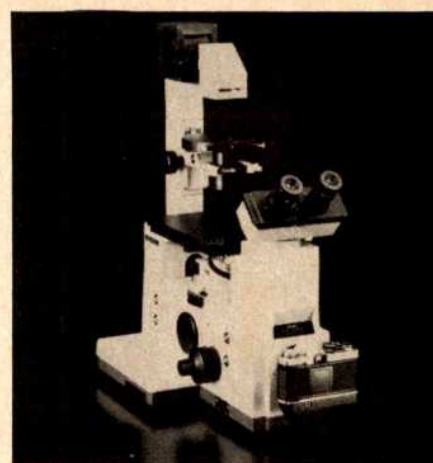
Bench-top freeze drying by FTS.

• The Tis-U-Dry is a bench-top freeze dryer which allows the histologist to freeze dry and fix heat-sensitive tissues in one chamber. The Tis-U-Dry series has condenser configurations to -130°C and a temperature controllable tissue platten to -100°C . A formaldehyde heater is connected directly to the freeze dry chamber and an optional resin embedding accessory that is mounted in a vacuum top plate allows all steps to be performed directly in the Tis-U-Dry, which is produced by FTS Systems Inc.

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• New from Paar Scientific is an economical microscope warm-stage which can be fitted easily to virtually any model of microscope. The device consists of the stage itself and a control box on which any temperature from ambient up to 60°C can be set quickly and easily to appear on a digital display. The stage measures 75 mm by 50 mm with a 6 mm aperture and the sample or specimen is protected from induced currents by a bifilar heating element specially constructed of a foil material which covers the entire stage area to prevent cold spots.

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The Olympus IMT-2.

• New from Olympus is the IMT-2 inverted research microscope specially designed for the examination of cell cultures. Attachments can be fitted to the IMT-2 for brightfield, phase contrast, differential interference contrast and fluorescent observations. Switch-over between these different observation methods is fast and simple, and a fixed stage system assures reliability during micro-manipulation. The microscope features an automatic exposure 35 mm camera with the facility to accept further camera attachments.

Reader Service No. 113.

• Two recent publications by Olympus Optical are available free of charge from Gallenkamp, the company's UK agents. The first, *Photomicrography*, is intended for scientists who have never taken a photograph down a microscope but also for experienced photomicroscopists who would like to improve their techniques. The second booklet, *Fluorescence Techniques in Microscopy* covers selection of fluorescence equipment, filters and methodology. Write to Olympus Department, Gallenkamp, Belton Road West, Loughborough LE11 0TR, stating which booklet is required.

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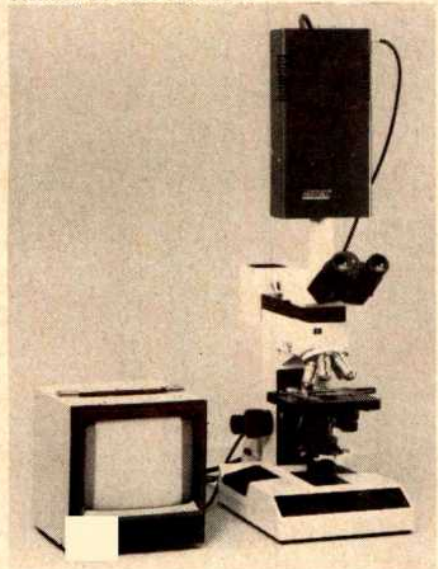
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• The Carl Zeiss Jena range of stereomicroscopes now includes the GSM, a light but robust stereomicroscope of high optical performance constructed following the Greenough principle. A twin lens slide allows rapid change of magnification and the extensive range of accessories allows various modes of illumination and measurement. The Zeiss Technival 2 is a high performance stereomicroscope with a five step magnification changer giving a 10:1 overall factor. It is a modular system giving a high degree of flexibility in magnification, lighting and photographic attachments. Most of the accessories for this instrument also suit the Citoval 2, a zoom stereomicroscope with a range of 10:1.

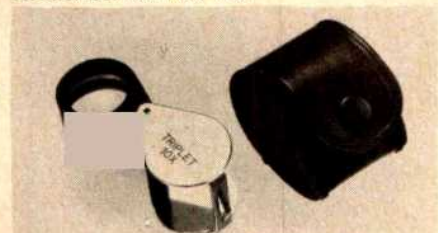
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Artek's new camera and monitor.

• Artek Systems Corporation has developed a 700-line camera and 700-line monitor for image analysis, dimensional measurement, particle analysis and visual inspection. A wide selection of image tubes is available for a variety of applications including easy viewing of microscopic samples. The camera and monitor system are compatible with Artek's image analysers and new Video Micrometer and Reticle Generator.

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Pocket magnification from Microtec.

• An extremely highly corrected 10× pocket magnifier has been added to the Microtec range of magnifiers. The lens construction is a new design which enables the high correction to be achieved with lenses over 20mm in diameter. The magnifier has a chromed brass frame and is supplied complete with a hide case.

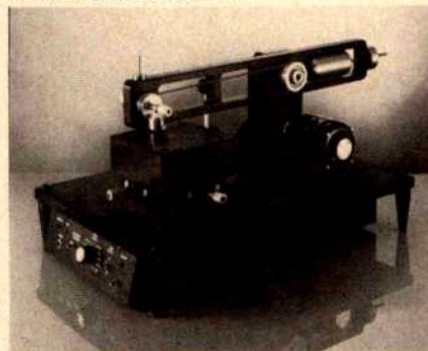
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The Vias workstation complete with digitizing tablet.

• Vias is a complete video image analysis workstation which includes both a flexible statistical package and a digitizing tablet. Featuring an easily accessible, completely menu-driven computer program, this system quickly and easily summarizes and performs complex morphometric calculations in any scale or coordinate system. Vias is available from Ted Pella Inc. of Tustin, California.

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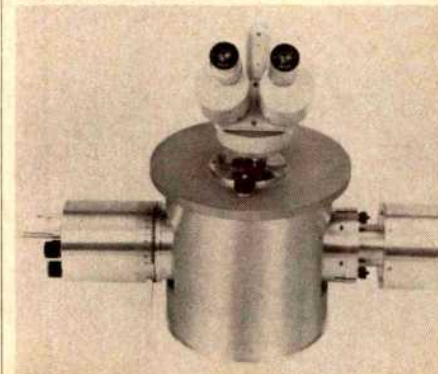
TEM preparation by 'dimpling'.

• The 'Dimpler' is a new prethinning instrument for TEM specimen preparation. Taking a 2.3mm or 3mm diameter specimen with a thickness of 100 micrometres or more, the VCR Dimpler D500 will delicately grind and polish a 'dimple' into the centre of the specimen, leaving a large area which is close to electron transparency. The perimeter of the disk is polished flat but remains in the form of a thicker rim so the specimen can be handled with more stability and greatly reduced risk of damage. It is common to achieve a 95% successful specimen yield. A variety of materials can be 'dimpled' — from the hard ceramics, glasses, quartz, sapphire and silicon, to the soft III-V semiconductor materials such as gallium arsenide or indium phosphide. The Dimpler Model D500 is available in Europe through Testbourne Ltd.

Reader Service No. 120.

• The Ion Tech FAB 306A fast atom bombardment specimen milling module extends the scope for specimen preparation techniques for both transmission and scanning electron microscope work. As well as milling conventional materials such as metals, ceramics and glasses, this new FAB equipped atom milling module enables more exotic materials to be thinned safely, thus extending the process into the latest semiconductor, polymer and biological technologies. Materials such as gallium arsenide, silicon oxides and many other very sensitive specimens — even delicate biological structures such as brain cells — can be processed in this new instrument. The FAB 306A combines improved wide dynamic range fast atom guns which produce beams from very low to high current density equivalents of charge free atoms with an efficient liquid nitrogen cooled specimen stage. Both charge and radiation damage are thus greatly reduced and a sensitive automatic terminator allows the milling process to be stopped instantly as desired. A wide beam atom gun facility also enables large area SEM specimens to be surface etched and removes smears and debris caused by mechanical polishing or other physical or chemical preparation techniques.

Reader Service No. 121.



TEM and SEM preparation using fast atoms.

Education's response to adversity

from Richard Pearson

The small-print in the UK government's recent review of the future of higher education is worthy of close examination.

THE UK government's long awaited 'Green Paper', *The Development of Higher Education into the 1990s*, has been widely criticized for its lack of originality and new ideas. But students, educationalists and employers alike will ignore it at their peril. If the final policy statement appearing in 1986 after the period of consultation follows the expected pattern, then many students may have their higher education aspirations frustrated, teachers will have increased workloads and decreased job opportunities, and while many employers will gain from the likely increase in the output of industrially relevant graduates, they may face a growing shortage of good generalists. Driven by the twin pressures of financial restraint and the demographic change which will significantly reduce the 18-year-old cohort over the next ten years, the paper sets out the government's expected priorities for higher education to the end of the century, a key priority being to make higher education more responsive to the needs of the economy.

It is important to get the demographic influence in perspective. There are two features that everyone agrees on. First, the major source of entrants to higher education will continue to be the 18-21-year-old population, and second, the size of this cohort will fall by one-third over the next decade, with most of this fall coming in the early 1990s.

In 1983 the Department of Education and Science (DES) published details of its then current thinking on the future demand for higher education. The report led to widespread comment and criticism from, amongst others, the Association of University Teachers, the Royal Society, the Royal Statistical Society and the Committee of Vice-Chancellors and Principals, who all agreed that DES had taken insufficient account of the rising proportion of women and mature entrants wishing to enter higher education, and the social class differential in the changing birth rate. In particular, it was noted that the birth rate for social classes I and II, which has provided nearly two-thirds of university entrants in the past, rose during much of the relevant period and as such the overall fall in student demand would not be as great as simple demographic trends suggested. There was also criticism that that no allowance had been made for policies that might increase the participation from working class families, or improve accessibility for mature students.

In the light of these criticisms, the DES forecasts were revised in 1984. The key figures are that the number of 18-year-olds in the population will fall from 914,000 in 1984 to a trough of 613,000 in 1995 before rising to 681,000 in the year 2000. Two projections of qualified demand are given by DES (Fig. 1). The projection, Variant X, assumes that student demand will continue at the levels prevailing in 1981-82, and that women will continue to catch up with men in their degree of participation in higher education. DES assumes, however, that the qualified demand index for women will

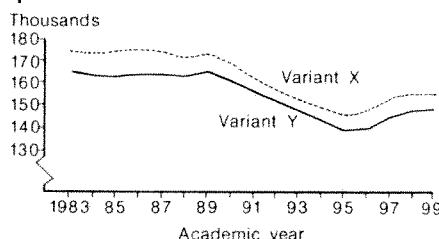


Fig. 1 Projections, from the DES report, of the total number of home initial entrants for the academic years 1983/4 to 1999/2000. Variant Y is the revised version.

not exceed 85 per cent of the observed level for men.

Variant Y, revised again in January 1985, assumes that student demand will continue at the levels observed in 1983, after the public expenditure cuts had caused some redistribution of higher education places. Both variants are based on assumptions about the prevailing real value of student grants and the influence of various social and economic circumstances, including labour market opportunities for graduates. DES concedes that, by 1999, actual student demand could be outside the limits set by Variants X and Y. The figures suggest a fall in student demand over the period to 1995 of about one-fifth.

For young home initial entrants to higher education (aged under 21 years), Variant X shows demand rising slightly to a peak of 134,500 in 1985 before falling back to 107,500 in 1994 and rising again to 117,700 in 1999. It is assumed here that the age participation index (API) will rise from 14.4 per cent of the age group to 16.9 per cent by 1999. Variant Y anticipated that young entrant demand had peaked in 1983-8, and that the peak age participation index assumed (for 1999) is 13.9 per cent of the age group.

Mature entrants have to be added to these totals, and the DES projections pro-

vide for a small growth from 37,500 in 1983-84 to about 40,000-43,000 mature entrants for the academic year 1989-90 and about the same number in 1994.

The government's current expenditure plans assume that expenditure will be available to meet a level of demand equal to variant Y. The University Grants Committee, interpreting these figures, has said that universities should plan on the assumption of a 2 per cent per annum cut in resources until 1990. This could be equivalent to the closure of a major institution each year.

A key theme of the green paper is meeting the needs of the economy which is seen to require a switch in places towards science and technology subjects. There is, however, some doubt whether suitably qualified and interested students will exist to fill the additional places. The paper finds it remarkable that the public sector institutions cannot fill their current places in science and technology subjects. Student interest in science and technology will therefore have to increase significantly over the next few years if it is to counteract the downward demographic trends and allow for the increased number of places to be filled.

Perhaps the most profound and unexpected part of the green paper is the appendix in which performance measures are proposed to help evaluate the effectiveness of individual institutions. Costs per student, years per graduate, cost per graduate, non-completion rates, entry qualifications, and initial labour market success are all factors to be measured. These in turn are consolidated into a 'rate of return' which, it is argued, can be used in the future to measure not only differences across the binary line, but also between institutions, and between subjects and departments within institutions. On the research side DES sees measuring outputs as rather harder, for example, in measuring the 'quality' of a book or an article, so it is working on ways of measuring inputs to research and their relationships to teaching.

Performance indicators may in due course prove to be even more controversial than the policy perspective, and it will be interesting to see how the debate about the future of higher education develops over the next year. □

Richard Pearson is at the Institute of Manpower Studies, Mantell Building, University of Sussex, Brighton BN1 9RF, UK.

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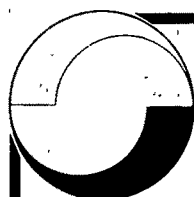
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Scriobh chugainn in nGaeilge mas fearr leat.

(7011)A

NEUROENDOCRINOLOGIST

Department of Physiology

QUEEN'S UNIVERSITY

A vacancy exists for an experienced neuroendocrinologist. The preferred applicant should have the Ph.D. and/or the M.D. degree, several years teaching experience and a record of independent research of high quality. The applicant will be expected to lead an active research program and to participate in undergraduate and graduate teaching. The facilities for research are outstanding and the appointment is a regular one under tenure regulations. The appointment will be made at a level appropriate to the candidate's qualifications.

Applications are encouraged from candidates of either sex. Applicants should submit a curriculum vitae, a brief statement of their research interests and objectives, selected reprints, and the names of three referees from whom a confidential assessment of research and teaching abilities may be obtained to: Dr. V. C. Abrahams, Department of Physiology, Queen's University, Kingston, Ontario, Canada K7L 3N6. (Second Advertisement).

(NW1870)A

THE DEPARTMENT OF PHYSIOLOGY

University of Manitoba

invites applications from young investigators in the field of

**CARDIOVASCULAR
 PHYSIOLOGY**

to be appointed at the level of Assistant Professor

The successful candidates will be expected to compete for local and national scholarship awards and develop their independent research programs. Applicants with backgrounds in membrane transport, electrophysiology, molecular biology or hemodynamics are encouraged to submit their curriculum vitae, an outline of their research interests as well as to arrange for three letters of reference to be sent to Dr. Henry G. Friesen, Professor and Head, Department of Physiology, Faculty of Medicine, University of Manitoba, Winnipeg, Manitoba, R3E 0W3 Canada. Deadline for application is 2 September 1985. The University of Manitoba is an Equal Opportunity/Affirmative Employer, but in accordance with Canadian Immigration, priority will be given to Canadian citizens and permanent residents of Canada.

(NW12)A

Marketing Manager Agrochemicals

c.£17,500 + car

Herts

This is a rare opportunity to run a small but growing agrochemicals business, operating within one of the most respected market leaders in the animal health field.

Reporting to the Divisional Director, your role will encompass all the classical marketing activities together with a very strong technical contribution. The responsibilities and demands of the position are both considerable and wide ranging – matched only perhaps by the personal opportunities and career potential.

Aged ideally 28-32, you will be a graduate in Biological or Agricultural Sciences. While your technical competence and credibility must be beyond question, it is essential that you also have some practical experience of and demonstrable aptitude for the commercial realities of the marketplace. Last, but by no means least, you must have the ambition and persuasiveness to drive the business forward, building soundly on its very strong base.

Call Richard Goldie on (0992) 552552 if an initial discussion would be helpful before writing to me, enclosing a brief CV together with an indication of recent salary progression, at Macmillan Davies Personnel Consultants, The Old Vaults, Parliament Square, Hertford, Herts. SG14 1PU.



(7052)A

Max-Planck-Institut für Biochemie Department of Membrane Biochemistry POST-DOCTORAL FELLOW IN MOLECULAR BIOLOGY

Applications will be accepted for a position in a group concerned with light-regulated gene expression in *Physarum*. Projects include cloning and sequencing of regulated genes as well as transcription in isolated nuclei/chromatin. Expertise in molecular biology techniques is essential. Preference will be shown to candidates having experience in lambda cloning.

The appointment is supported by the Deutsche Forschungsgemeinschaft and will be for 18 months starting as soon as possible. The salary will be in the range of DM 2000,—. Applications together with CV, research experience and interest, list of publications and the name of two referees should be sent to Dr. Thomas Schreckenbach, Max-Planck-Institut für Biochemie, 8033 Martinsried, FRG before 30th August. (W1990)A

UNIVERSITY OF THE SAARLAND (FRG), DEPARTMENT OF PHYSIOLOGY

TWO POSTDOCTORAL POSITIONS

to work on the electrophysiology of single myelinated nerve fibres (voltage clamp, fluctuation analysis, gating currents).

The project is supported by two project grants from the DFG. The appointments are from 1st January 1986 to 31 December 1987, with possible prolongation. The salary is about DM 55,000 — DM 65,000 per annum, depending on age and family status.

Applications (including a curriculum vitae and the names of two referees) should be sent to Prof. H. Meves or Prof. B. Neumcke, I. Physiologisches Institut der Universität des Saarlandes, D — 6650 Homburg/Saar, Federal Republic of Germany. (W1994)A

UNIVERSITY OF LONDON Royal Holloway and Bedford New College

Readership in Environmental Systems Analysis and Control (within the Department of Geography)

The Senate invite applications for the above post from established researchers to initiate and develop research and teaching in environmental systems analysis using dynamical modelling techniques. Applications (10 copies) should be submitted to the Academic Register (N), University of London, Malet Street, London WC1E 7HU, from whom further particulars should first be obtained.

Closing date for receipt of applications — 16 August 1985. (7012)A

UNIVERSITY OF QUEENSLAND READER IN SOIL PHYSICS (tenured) — AGRICULTURE

To teach Soil Physics at both undergraduate and postgraduate level, supervise postgraduate students and develop research. Applicants should have a Ph.D. in Soil Physics and considerable experience in teaching and research in Soil Physics. Experience with Australian soils, in particular Vertisols, an advantage.

Salary per annum: \$A43,929

Closing date: 14th June, 1985. Ref. 14185.

Additional information and application forms are available from the Secretary-General, Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF.

The University of Queensland is an Equal Opportunity Employer. (W1989)A

CSIRO

AUSTRALIA

MOLECULAR BIOLOGIST

\$26,001 — \$38,243

DIVISION OF PROTEIN CHEMISTRY

PARKVILLE VIC, AUSTRALIA

FIELD: Molecular Biology/Genetic Engineering.

GENERAL: The Division of Protein Chemistry conducts scientific research on the structure and function of proteins and their production via biotechnology and synthetic routes. It has a total staff of 116. The appointee will be a member of the Program Applied Protein and Gene Technology which has 22 professional scientists working on biotechnology based projects. Current research projects within the Program include the production of subunit and peptide vaccines, diagnostic probes, and the genetic improvement of industrial microorganisms. Besides recombinant DNA techniques, scientists in the Division have expertise in protein and peptide chemistry, immunochemistry, X-ray crystallography, organic chemistry, cell biology and electron microscopy. The Division is very well equipped and has excellent facilities for recombinant DNA research. The Division is situated in close proximity to some of the major bio-medical research institutes in Australia: Melbourne University, CSIRO Division of Animal Health, and the Commonwealth Serum Laboratories. There is excellent potential for collaboration with scientists in the above institutions and elsewhere both within and outside the CSIRO. Adequate technical assistance will be provided to the applicant.

DUTIES: The appointee will join a multidisciplinary research team involved in the production of significant new biological products focussing initially on a vaccine for an important disease of poultry.

Specific duties would include the construction and modification of useful expression vectors for the production of the avian antigen vaccine and other high value proteins in microbial systems such as yeast, *E. coli* and other bacteria.

QUALIFICATIONS: A PhD or equivalent qualification and post-doctoral experience in a relevant field, and demonstrated research ability. Experience in recombinant DNA research is essential. Applicants should preferably have prior experience in the construction and use of yeast and/or bacterial expression vectors.

TENURE: Indefinite with Australian Government superannuation benefits available. However, applicants who would prefer a fixed term appointment of 3-5 years will be considered.

NOTE; Indefinite Appointment attracts assistance with travel and removal costs for appointee and immediate family.

Term Appointment attracts these benefits plus assistance with relocation costs at the end of the term.

APPLICATIONS: Stating relevant personal particulars, including details of qualifications and experience, the names of at least two professional referees and quoting reference number A2488, should be directed to:

The Chief
CSIRO Division of Protein Chemistry
343 Royal Parade
PARKVILLE VIC 3052

one month from date of publication
CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER
(W2004)A

THE UNIVERSITY OF LANCASTER

Department of Environmental Science

ENVIRONMENTAL RADIOACTIVITY

Applications are invited for the post of Research Associate to work with Dr. J. Hamilton-Taylor and Dr. M. Kelly on a study of transuranic elements in the estuarine environment. Ideally, applicants should possess Ph.D. and have research experience in environmental chemistry or geochemistry, although candidates with alternative qualifications will be considered. The appointment would be for six months from 1 October 1985, although funds for additional periods have been applied for. Salary up to the £10,330 point of Research 1A scale (£7,520 — £12,150 under review).

For further details and application form, please write to the Establishment Office, (quoting reference LSSO/B) University House, Bailrigg, Lancaster LA1 4YW where applications (five copies) naming three referees should be sent to arrive NOT LATER THAN 1 August 1985.
(7047)A

LEONHARD PROFESSORSHIP IN SOLID STATE DEVICE ENGINEERING

Applications and nominations are invited for a recently endowed professorship in Solid State Device Engineering within the Department of Electrical Engineering at The Pennsylvania State University. Applicants should have an earned doctorate in electrical engineering, engineering physics, or a related field, and have demonstrated the ability to conduct research and teaching in the field of Solid State Device and Microelectronics. Strong leadership qualities are also emphasized.

The successful applicant is expected to join the existing effort in solid state device research and help expand and develop it into one of prominence in both research and instruction. The College of Engineering has made substantial commitment to support this program including updating of the laboratory facilities and new faculty hiring.

Currently, research in the solid state device and related materials area includes research in silicon device processing, such as plasma and reactive ion etching, thermal nitridation of silicon and various oxide research including ultra-thin oxides, and defect control. In addition, research in III-V device area includes material defects, electrical noise and performance improvement of micron and sub-micron structures.

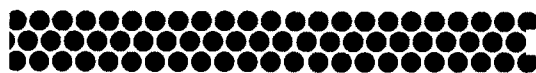
Combined research in the solid state device area typically amounts to about one million dollars annually. Collaborative research opportunities exist with the Materials Science department, Engineering Science and Mechanics department, the Chemistry and the Physics departments and the Materials Research Laboratory at Penn State.

Résumés, nominations, or inquiries should be directed to the following address by no later than September 15, 1985, or until a suitable candidate is selected.

Chairperson, Leonhard Professor Search Committee
Department of Electrical Engineering
Box J, 129 Electrical Engineering East
The Pennsylvania State University
University Park, PA 16802

An Equal Opportunity/Affirmative Action Employer.

(NW1910)A



The European Molecular Biology Laboratory, an international research institute situated in Heidelberg, West Germany, invites applicants for two vacancies in the protein crystallography group headed by Dr. K.S. Wilson at the Laboratory's Outstation at the Deutsches Elektronensynchrotron (DESY) site in Hamburg

(PROTEIN) CRYSTALLOGRAPHERS/ PHYSICIST

The Outstation has developed advanced instrumentation for the use of synchrotron X-ray radiation in structural biology: protein crystallography, fibre and solution diffraction, X-ray spectroscopy (EXAFS)

The facilities are used by many groups mainly from the Member States. The candidates will be expected to engage in research projects, mostly on a collaborative basis with outside groups, which make use of the outstanding properties of synchrotron radiation (high intensity, wavelength tunability, polarization and time structure) for protein crystallography. Besides these activities, they will also assist outside groups with protein crystallographic data collection.

For the first position, experience in experimental physics or crystallography is required as the successful candidate will be responsible for the instruments for protein crystallography at the Outstation.

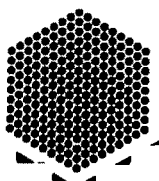
For the second position, the emphasis will be on the development of software for data collection with electronic area detectors. Experience in real time programming of instruments like four-circle diffractometers will be an asset.

An above-average salary will be offered to the successful candidate. Certain allowances are payable in addition, depending on personal circumstances. An initial contract of three years' duration, which can be renewed, will be offered.

Please write briefly for an application form, quoting reference No 84/8 & 9 to

EMBL,
Personnel Section,
Postfach 10.2209,
D-6900 Heidelberg

(W2008)A



EMBL



THE WORLD HEALTH ORGANIZATION
invites applications for the post of
**SCIENTIST (RESPONSIBLE OFFICE, RESEARCH
AND DEVELOPMENT)**

with its Special Programme for Research and Training in Tropical Diseases, Geneva. The incumbent will coordinate the strategic planning of the Research and Development operations of the Special Programme for Research and Training in Tropical Diseases (TDR) and the execution of the Research and Development plans as recommended by the Scientific and Technical Advisory Committee (STAC) and approved by the Joint Coordinating Board (JCB), and provide secretariat services for meetings of the STAC and its sub-committees; coordinate the planning of individual Scientific Working Groups (SWGs) and exploit opportunities for synergy, maintain programme balance and cohesion and avoid duplication among the groups; monitor and evaluate effectiveness and efficiency of research and development undertaken by SWGs.

Qualifications and Experience: A degree in medicine or a biomedical science degree at the doctorate level from a medical school or a university of recognized standing. Research qualifications to a doctorate level in association with medical degree or post-doctoral qualifications if primary doctorate degree is non-medical. At national level, at least 10 years' experience in biomedical research, including: (a) independent contributions to scientific knowledge; (b) direct involvement in research and authorship of a substantial number of scientific publications in refereed journals; (c) directing a research group; (d) research administration and participation in the work of research funding bodies; (e) teaching and training of researchers, both through the organization of training courses and the supervision of doctorate students. At international level: 5 years' experience in the planning and organization of research in an international framework. Excellent knowledge of English or French and a working knowledge of the other language.

The salary scale ranges from US\$ 42,594 (single) and from US\$ 46,125 (with dependents) net of tax per annum. Applications are invited from candidates of either sex — applications from women are encouraged. Qualified candidates should send their detailed curriculum vitae not later than mid July 1985 quoting "TDR/85" to:

Personnel (MPR), World Health Organization, CH — 1211 Geneva 27

Only those candidates under serious consideration will be contacted.

(W1984)A

**UNIVERSITY OF QUEENSLAND
LECTURER IN PHARMACOLOGY
(fixed term) — PHYSIOLOGY &
PHARMACOLOGY**

Graduates in Science, Pharmacy, Medicine, Dentistry or Veterinary Science but must have additional experience in Pharmacology i.e., a higher degree in Pharmacology and/or significant postdoctoral research experience and/or teaching experience in Pharmacology.

Salary per annum: \$A26,236 — \$A34,467.

Closing date 20th September, 1985. Ref. 27385.

Additional information and application forms are available from the Secretary-General, Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF.

The University of Queensland is an Equal Opportunity Employer.
(W1992)A

**POSTDOCTORAL POSITIONS
HOWARD HUGHES MEDICAL INSTITUTE/
PHILADELPHIA**

Fully funded postdoctoral positions in molecular biology and molecular genetics are now available at the Howard Hughes Medical Institute of the University of Pennsylvania. Active research projects include: transcriptional and translational control of eukaryotic gene expression, mechanisms of cellular transformation and gene amplification, molecular mechanisms of chromatin inactivation and the application of molecular genetics to studies of inherited disease.

For information please contact:

Dr. Steve Liebhaber, Coordinator
Howard Hughes Medical Institute
Department of Human Genetics
Medical Labs Building/G3
University of Pennsylvania School of Medicine
Philadelphia, PA 19104

(NW1869)A

**UNIVERSITY OF ULSTER
'NEW BLOOD' LECTURESHIP IN
EXPERIMENTAL SOLID STATE PHYSICS**

at Coleraine, Ref: C85/126

To undertake research in the physics of low-dimensional systems, surface physics spectroscopies or low temperature (millikelvin) physics or in some other area of condensed matter physics, and to contribute to the teaching in the Physics and Electronics programmes of the Department of Physics.

Applicants should hold a good honours degree and a higher degree in Physics and should have had appropriate research experience. In accordance with the New Blood appointments scheme, applicants should normally be under the age of 35 years and should not hold permanent university appointments in the United Kingdom.

Salary will be determined by age, qualifications and experience within the range £7,520 — £14,925 per annum (under review).

Further details are available from the Staffing Office, University of Ulster at Coleraine, Cromore Road, Coleraine, Co Londonderry BT52 1SA (Telephone Coleraine (0265) 4141, Ext 225. Telex 747597) to whom applications, including a full curriculum vitae and the names and addresses of three referees, should be sent not later than 2 August 1985. (6993)A

THE MAX-PLANCK-GESELLSCHAFT
invites applications for the position of a
RESEARCH GROUP LEADER

from promising young scientists who have achieved a degree of international recognition in their field, preferably in

Cell Biology, Developmental Biology, or Neurobiology

The successful applicant will be expected to conduct a vigorous, original and independent research programme at the

Friedrich-Miescher Laboratory in Tübingen

The position is for 5 years, carries a salary of 65-70,000 DM p.a. (C3 scale of the Bundes-Besoldungsgesetz) and is roughly equivalent to an Assistant Professorship (US) or University Lectureship (UK). There is no teaching commitment.

The group will have modern laboratory facilities, operating expenses, and funding for scientific and technical co-workers.

Applications, including a tabular curriculum vitae, list of publications, and a description of major scientific achievements and future research plans, should be submitted until end of September 1985 to:

Max-Planck-Gesellschaft, zur Förderung der Wissenschaften e.V., — Generalverwaltung —, Postfach 647, D-8000 München 1. (W1991)A

CSIRO

AUSTRALIA

RESEARCH FELLOW

VIROLOGIST OR

MOLECULAR BIOLOGIST

\$A26,001 — \$32,083

INSTITUTE OF ANIMAL AND

FOOD SCIENCES

CANBERRA ACT

GENERAL: A joint CSIRO-ANU project has been established within the Department of Microbiology, The John Curtin School of Medical Research, Australian National University, to construct recombinant poxviruses expressing foreign antigens and to assess immunological responses to such antigens. The use of recombinant poxviruses as vaccines for human and veterinary use is being explored.

DUTIES: The appointee will undertake research on the virology and molecular biology of avian poxviruses with a view to developing techniques for the construction of avian poxviruses as vectors for poultry vaccines. Techniques for the construction of recombinant vaccinia viruses are already established. Collaboration with members of CSIRO already within the Department of Microbiology and other groups will be encouraged.

QUALIFICATIONS: A PhD degree or equivalent qualifications in virology and/or molecular biology or related discipline.

TENURE: A term of three years with Australian Government superannuation benefits available.

APPLICATIONS: Stating relevant personal particulars, including details of qualifications and experience, the names of at least two professional referees and quoting reference No A7287, should be directed to:

The Director
Institute of Animal and Food Sciences
CSIRO
PO Box 225
DICKSON ACT 2601

by 2 August 1985
CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER
(W2001)A

FACULTY POSITIONS IN

ELECTRICAL ENGINEERING

(SOLID STATE DEVICE AND

MICROELECTRONICS AREA)

The Pennsylvania State University at University Park, Pennsylvania invites applications for tenure track faculty positions at the Assistant Associate and Full Professor ranks in Electrical Engineering in the area of Solid State Device and Microelectronics. Applicants should have an earned doctorate in electrical engineering, engineering physics or related fields, and have demonstrated the ability to conduct research and teaching in the field of Solid State Device and Microelectronics.

The successful candidates are expected to join the existing effort in the solid state device research and help develop it into one of prominence. The College of Engineering has made substantial commitments to support this program by hiring of an endowed professor in this area.

Currently, research in the solid state device and related materials area includes research in silicon device processing, such as plasma and reactive ion etching, thermal nitridation of silicon and various oxide research including ultra-thin oxides, and defect control. In addition, research in III-V device area includes material defects, electrical noise and performance improvement of micron and sub-micron structures.

Combined research in the solid state device area typically amounts to about one million dollars annually. Collaborative research opportunities exist with the Materials Science department, Engineering Science and Mechanics department, the Chemistry and the Physics departments and the Materials Research Laboratory at Penn State.

Résumés, or inquiries should be directed to the following address by no later than September 15, 1985, or until suitable candidates are identified.

Advisory Committee
Department of Electrical Engineering
Box H, 129 Electrical Engineering East
University Park, PA 16802

An Equal Opportunity/Affirmative Action Employer. (NW1909)A

DEAN

FACULTY OF HEALTH SCIENCES

McMaster University invites applications or nominations for the position of Dean, Faculty of Health Sciences. This appointment is to be effective 1 July, 1986 or as negotiated. The term of appointment is for five years with the possibility of renewal for an additional five years.

The successful candidate will possess appropriate academic qualifications, demonstrated leadership ability and a record of effective administrative experience. The Faculty of Health Sciences consists of the School of Medicine and Nursing and offers programmes in Physiotherapy and Occupational Therapy.

Applications/nominations, accompanied by a detailed curriculum vitae and names of three referees, should be forwarded to: **Dr D.R. McCalla, Vice-President, Faculty of Health Sciences, McMaster University, 1200 Main Street West, Hamilton, Ontario, Canada L8N 3Z5.** In accordance with Canadian immigration requirements, this advertisement is directed to Canadian citizens and permanent residents.

(NW11)A



MCMASTER UNIVERSITY

HAMILTON, ONTARIO, CANADA.

THE UNIVERSITY OF

MANCHESTER

RESEARCH ASSISTANT IN

MEDICAL GENETICS

Applications are invited for this post to work on a project investigating the organisation of class I HLA genes. A good background in molecular biology is required and preference will be given to candidates with experience of recombinant DNA techniques. Initial salary range pa: £6,600 — £7,520. Superannuation.

Applications, with curriculum vitae and the names of two referees, should be sent to Dr T Strachan, Department of Medical Genetics, St Mary's Hospital, Manchester M13 0JH. (6988)A

UNIVERSITY OF

ST ANDREWS

DEPARTMENT OF PHYSIOLOGY
AND PHARMACOLOGY

Applications are invited for a
POSTDOCTORAL
APPOINTMENT

To work on an electrophysiological study (using voltage — and patch-clamp techniques) designed to investigate the role of phosphorylation-dephosphorylation reactions to the transmitter action of 5-hydroxytryptamine in identified neurones. Some experience of electrophysiological techniques would be useful. The appointment is funded by the wellcome trust and is for 3 years, starting salary within range £7,520 — £7,980 per annum, plus USS.

Applications (two copies preferably in typescript) with the names of two referees should be sent to the establishments officer, The University, College Gate, St Andrews, Fife, KY16 9AJ, to arrive not later than 23 July 1985. (7029)A

UNIVERSITY OF OXFORD

PROFESSORSHIP OF THE

PHYSICS AND CHEMISTRY

OF MINERALS

The electors intend to proceed to a election to the newly established Professorship of the Physics and Chemistry of Minerals in the Department of Earth Sciences. The stipend of the professorship is at present £20,795.

Applications (ten copies, or on from overseas), naming three referees but without testimonials should be received not later than 2 August by the Registrar, University Offices, Wellington Square, Oxford OX1 2JD, from whom further particulars may be obtained. (7015)A

UNIVERSITY OF SUSSEX

School of Biological Sciences

POST-DOCTORAL
RESEARCH FELLOW:
DEVELOPMENTAL
GENETICS

Geneticist or developmental biologist required for a research programme to identify the genetic events involved in the onset of polyteny during cellular diversification in *Drosophila* embryogenesis

This is a two year appointment financed by the Science and Engineering Research Council. Start date as soon as possible.

Starting salary in the range £7,520 — £8,920 on the scale for Research Fellow Grade 1A £7,520 — £12,150 with superannuation benefits.

Applications with full curriculum vitae to Dr J R S Whittle, School of Biological Sciences, University of Sussex, Falmer, Brighton BN1 9QC from whom further information can be obtained (Telephone 0273 60675 Ex 467) (7004)A

Allelix

HEAD OF PLANT BIOLOGY

Allelix — a major established Canadian biotechnology company — is seeking an accomplished scientific manager to direct multi-disciplinary research and development programs for its agricultural programs.

This position involves overall responsibility for planning, leading and managing up to 40 scientists applying new techniques in biotechnology to varietal development and crop improvement.

We are seeking a scientific manager whose innovative impact on crop development is demonstrated by a record of accomplishment in scientific research management and product development.

Allelix has a broad base in all major biotechnology disciplines and is well financed by three large Canadian organizations. Research and development is conducted at modern, well-equipped laboratories near Toronto and in a 100-acre field station.

Interested candidates are invited to send résumés, quoting file number PB002, to:

Dr. D.C. Burke
Vice-president & Scientific Director
Allelix Inc.
6850 Goreway Drive
Mississauga, Ontario
Canada L4V 1P1

(NW10)A

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF PATHOLOGY TECHNICIAN GRADE 4

to prepare histological sections for research and teaching. Training for specialist techniques needed will be given.

Candidates must possess ONC or appropriate equivalent as minimum qualification plus seven years histology experience.

Salary within range £6,106 — £7,024 per annum (under review).

Application forms may be obtained from the Registrar, the University, PO Box 147, Liverpool, L69 3BX.

Quote Ref: RV/134/N. (7022)A

UNIVERSITY OF ST ANDREWS

DEPARTMENT OF ZOOLOGY Applications are invited for a post of GRADUATE RESEARCH ASSISTANT

to work on the developmental biology of *Drosophila* imaginal discs, using the techniques of tissue culture and ultrastructure. Candidates should have a first or second class Honours degree in Biology or Zoology. Some knowledge of developmental or cell biology and practical experience of transmission electron microscopy, would be an advantage. This post is funded by the Science and Engineering Research Council for approx 12 months. Salary within range £6,600 — £7,055 per annum, plus USS.

Applications, with full curriculum vitae and names of two referees, should be sent as soon as possible to Dr M J Milner, Department of Zoology, Bute Buildings, The University, St Andrews, Fife, KY16 9TS. (6996)A

IMPERIAL COLLEGE (University of London) SUBMICRON LITHOGRAPHY

We are seeking a scientist/engineer who has recently obtained a doctorate to work on electron beam stimulated decomposition of inorganic solids for use as resists in sub-micron lithography. A strong ability in experimental work plus a facility with programming is highly desirable. The appointment is, in the first instance, for one year at a stipend of £8,753 (this includes London Weighting).

Write to Professor Mino Green, Dept of Electrical Engineering, Imperial College, Exhibition Road, London, SW7 2BT. (7024)A

CAMBERWELL HEALTH AUTHORITY King's College Hospital CYTOGENETICIST

DEPARTMENT OF HAEMATOLOGY

Salary: £7,021 — £9,395 pa inc (depending on degree).

Required to perform cytogenetic analysis on samples from patients with leukaemias, lymphomas and related haematological disorders. The Department provides a cytogenetic service to haematologists throughout the South East Thames Region. Previous experience of cytogenetics of leukaemias would be an advantage. An appropriate science degree is essential.

For further details, contact Mr G J Mufti or Professor A J Bellingham, 01-274 6222 extension 2013 or 2045. Application forms and job description available from Unit Personnel Department, King's College Hospital, Denmark Hill, London SE5 9RS, telephone 01-274 6222 extension 2751/2254. (7026)A

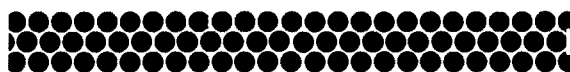
WASHINGTON UNIVERSITY ST. LOUIS, MISSOURI POSTDOCTORAL POSITIONS

Interactive and broadly based senior research group interested in biological problems associated with BONE and BONE MARROW CELLS invited well qualified, recent doctoral graduates to apply for laboratory positions at the postdoctoral level. Research areas of particular interest to the group include:

1. Hormonal and immune regulation of bone remodeling
2. Mechanisms of collagen and bone matrix degradation
3. Gene expression in bone forming and resorbing cells
4. Mitogenic and chemotactic properties of thrombin and other clotting proteins.
5. Origin and differentiation of osteoblasts and osteoclasts
6. Vitamin D in cell maturation and immune function

Ideal candidates should have some prior laboratory experience with both contemporary biochemical and cell biological techniques. Partial and full stipends are available depending upon the citizenship status of the individual applicant.

Interested parties should submit a curriculum vitae, the names and addresses of three references, and a brief statement of research experience and goals to: Prof. George Wilner, Box 8050, Washington University School of Medicine, St. Louis, Missouri 63110, USA. (NW1)A



The European Molecular Biology Laboratory, an international research institute situated in Heidelberg, West Germany, invites applicants for two vacancies in the protein crystallography group headed by Dr. K.S. Wilson at the Laboratory's Outstation at the Deutsches Elektronensynchrotron (DESY) site in Hamburg

TECHNICAL ASSISTANTS

Both posts are in the protein crystallography group at the Hamburg outstation, to be headed by Dr. Keith Wilson. The assistants will be active both in the support of users at the outstation and in the scientific research or the group.

For one position a basic training in engineering or physics and for the second post experience in crystallography/biochemistry is required.

For both positions some knowledge of computer programming would be an advantage.

A working knowledge of English is essential. A second language (French or German) is desirable.

An above-average salary will be offered to the successful candidate. Certain allowances such as family, children's and non-resident allowance will be payable in addition, depending on personal circumstances.

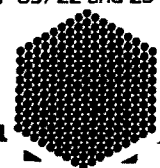
An initial contract of 3 years' duration will be offered to the successful candidate. This can be renewed, depending on circumstances at the time of review.

Please write briefly for an application form, quoting reference N° 85/22 and 23 to:

EMBL,
Personnel Section,
Postfach 10.2209,
D-6900 Heidelberg.

(W2009)A

EMBL



SEARCH SCIENTISTS

Agricultural R&D



Letcombe Manor, Oxfordshire already has a track record in agricultural developments. Until recently it's been a research centre for the Agricultural Research Council. Now it's owned by the Dow Chemical Company Limited. We are investing a considerable amount in re-equipping the Manor to provide the most advanced facilities for our expanding European Agrochemical R&D operation.

Letcombe Manor gives us much needed room for growth. And as a first step we are also looking to invest in the following high calibre professionals:

R&D CHEMISTS

— to join a team undertaking work in any of the following:

- * Formulation Research & Development
- * Formulation Analysis
- * Residue and Environmental Analysis

Qualified to degree level or equivalent in Chemistry or a relevant discipline. Ref: 100.

PLANT PATHOLOGIST

— to form and lead a team involved in creating and developing both laboratory and glasshouse test procedures designed to identify new commercial fungicides.

You should have a minimum of 5 years' post doctoral experience, preferably in pesticide research. Ref: 101

BIOCHEMIST

— to join a small, innovative team researching plant pathogenic fungi.

You should be qualified to at least degree, preferably PhD level, with proven success in biochemical research. Ref: 102.

CROP PROTECTION

— Biologist or Biochemist with a good Honours degree

to the ~~Minor~~ borne



or PhD to join a team developing crop protection herbicides. Ref: 103.

REGISTRATION SPECIALIST

— for the preparation, submission and negotiation of our agrochemical product registration in Europe and Africa.

We'll be looking for someone experienced in any area of agrochemical research and with a willingness to train in registration work — ideally the person should have a degree or equivalent in chemistry or a relevant discipline. Ref: 104

In all cases we will be looking for highly self-motivated individuals with the personal and professional skills to develop their career with Dow, plus the enthusiasm, flexibility and commitment to make a

positive contribution within a small team environment.

The benefits of working for Dow go far beyond the superior facilities of our new R&D Centre. Indeed prospects for the future, in terms of our growth and your career development are excellent — there is no limit for individuals of calibre to progress. Naturally, rewards are also very generous, with competitive salaries and a wide range of fringe benefits, including generous relocation assistance where appropriate.

For more information about careers within our new Research facility, please telephone or write, quoting the appropriate reference number, to Liz Sim, Personnel Officer, Dow Chemical Co. Limited, Crossbank Road, King's Lynn, Norfolk PE30 2JD. Tel: King's Lynn (0553) 761251



Let ~~us~~ ~~be~~ ~~the~~ ~~Minor~~

*Trademark of The Dow Chemical Company

CSIRO AUSTRALIA PHYTOPLANKTON ECOLOGIST

\$A26,001 — \$A38,243 p.a.
DIVISION OF FISHERIES RESEARCH
MARINE LABORATORIES
HOBART TAS

GENERAL: The Division of Fisheries Research is a major component of the CSIRO Marine Laboratories, which is Australia's principal marine research institution. The Division currently employs about 80 scientists and 55 support staff in laboratories in Hobart, Perth and Brisbane.

DUTIES: To carry out research into phytoplankton species identification and photosynthetic pigments for field programmes designed to expand the knowledge of phytoplankton in Australian waters from tropical, sub-tropical and temperate regions. Responsible for in-shore and off-shore field programmes investigating phytoplankton blooms and successions, red tides and mariculture applications.

QUALIFICATIONS: A PhD degree or equivalent qualifications preferably in phytoplankton ecology with knowledge of the phytoplankton of Australian tropical and temperate waters and skills in electron microscopy and phytoplankton pigment techniques. Ability to work effectively at sea essential.

TENURE: Indefinite with Australian Government superannuation benefits available.

APPLICATIONS: Stating relevant personal particulars, including details of qualifications and experience, the names of at least two professional referees and quoting reference No A2361, should be directed to:

The Chief
CSIRO Division of Fisheries Research
GPO Box 1538
HOBART TAS 7001

by 2 August 1985
CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER
(W2002)A

General Anatomy

As Lecturer in the Department of General Anatomy in the School of Dentistry you will be required to teach human anatomy and human biology to dental and science students and to engage actively in research.

You should have a degree in dentistry, medicine or science, postgraduate experience being an added recommendation. Areas of research interest include developmental biology, human biology and comparative and forensic anatomy. Duties are to be assumed on 1 June 1986.

Salary: R16,257 — R27,648 per annum. **Benefits:** • pension • medical aid • annual bonus • remission of fees • generous leave • housing subsidy, travel, removal and settling-in allowances (if eligible).

It is the policy of the University not to discriminate on the grounds of sex, race, colour or national origin.

For a detailed information sheet contact:

Mr Geoff Henning, South
African Universities Office,
Chichester House, 278
High Holborn, London
WC1V 7HE, England.
Submit your application by
30 September 1985.



UNIVERSITY OF
THE WITWATERSRAND

UNIVERSITY OF SURREY DEPARTMENT OF MICROBIOLOGY POST DOCTORAL RESEARCH FELLOW

In this Department we have an active and expanding research group, under Dr J W Dale, which is applying recombinant DNA techniques to a variety of problems associated with mycobacterial diseases such as leprosy and tuberculosis. We are seeking to appoint a Research Fellow, preferably at post doctoral level or with other experience of DNA cloning. The person appointed will be responsible for a specific aspect of the project, involving the cloning and expression in *E coli* of genes for key mycobacterial antigens.

The post is available as soon as an appointment can be made, and is for two years. The starting salary will be up to £9,390 per annum according to age, experience and qualifications.

Applications in the form of a curriculum vitae (3 copies), including the names of two referees, should be sent to the Personnel Officer (JLG), University of Surrey, Guildford, Surrey, GU2 5XH by 19th July 1985 quoting reference 403. Further details can be obtained from Dr Jeremy Dale, telephone (0483) 571281 ext 871/369/N. (7001)A

LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE

DEPARTMENT OF CLINICAL TROPICAL MEDICINE MOLECULAR BIOLOGIST

This is a position funded by the Wellcome Trust for 3 years leading to a tenured post thereafter. The Wellcome position includes funding for a technician. The School has provided considerable newly-renovated and equipped laboratory space for the Department of Clinical Tropical Medicine. The present research interests in this department include the immunology of leprosy aiming towards the development of a vaccine for leprosy and a study of acute phase proteins synthesised by the liver following acute inflammatory disease. Candidates for this position must have post-doctoral experience in recombinant DNA technology and would be encouraged to pursue their own interests in addition to collaboration on current projects in the department. The new laboratories are adjacent to the Wolfson Unit of Molecular Biology with which there will be close contact.

An appointment will be made on the Universities' Lecturer or Senior Lecturer scale according to the qualifications and experience of the person appointed with membership of USS.

Candidates should send full curriculum vitae and names of three professional referees to the Personnel Officer at the London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, quoting reference CTM7. Closing date Monday 5 August 1985. (7038)A

UNIVERSITY OF BIRMINGHAM Faculty of Science DEPARTMENT OF SPACE RESEARCH

Applications are invited for a POSTDOCTORAL RESEARCH FELLOW

to undertake data analysis and interpretation of the results from the Hard X-ray Imaging Spectrometer on the Solar Maximum Mission spacecraft. Collaborative work with other data sets from this mission is encouraged.

Post is tenable for up to two years and is funded by SERC. Applicants should have a PhD in Physics or Astronomy.

Salary on the Research Fellow scale £7,520 — £12,150 plus superannuation.

For further information phone 021-472-1301, ext 2559 quoting reference E1.

No formal application form. Three copies of application, including full curriculum vitae and naming three referees to Assistant Registrar (Science) PO Box 363, Birmingham B15 2TT, by Thursday 18th July, 1985. (7016)A

UNIVERSITY OF SUSSEX School of Biological Sciences

Applications are invited for two posts funded by the MRC to work on an interdisciplinary project entitled "Influence of carboxylic acids and heavy metals on the behaviour and physiology of pulmonate snails of medical importance" under the direction of Dr J D Thomas:—

i A POST-DOCTORAL RESEARCH FELLOW (RESEARCH OFFICER IA) ii TECHNICIAN GRADE 5

Applicants should preferably have experience with HPLC, AAS, use of radiolabel and toxicity testing.

The posts are tenable to the end of March 1986 in the first instance, and are to commence as soon as possible. Salaries on the Research Officer IA scale: £7,520 to £9,860. ii Technician Grade 5 scale: £6,581 to £7,011.

Two copies of the application, including full curriculum vitae and the names of three referees should be sent to Dr J D Thomas, School of Biological Sciences, University of Sussex, Falmer, Brighton BN1 9QG. (7003)A

Please mention

nature

when replying to
these advertisements

THE UNIVERSITY OF PAPUA NEW GUINEA

Port Moresby

LECTURER/SENIOR LECTURER IN ENVIRONMENTAL AND ANALYTICAL CHEMISTRY — PO31006/85

The Chemistry Department has a vacancy from the beginning of 1986 in the field of Environmental and Analytical Chemistry. The appointee will be required to teach a final year course in Environmental Chemistry, a more general course in Analytical Chemistry and contribute to basic and honours level courses. Applicants should have research skills which will allow involvement with research programmes in inland water quality and coastal water quality sponsored by the South Pacific Regional Environmental Programme.

The appointment will be for two years, with a possible extension for one further year overlapping with a national member of staff who will return from training overseas in early 1988. The University welcomes applications from persons who would be on secondment from a permanent position.

SALARY: Senior Lecturer K21255, Lecturer Grade 2 K19405, Lecturer Grade 1 K17555 per annum plus gratuity.

OTHER CONDITIONS: The gratuity entitlement is based on 24% of salary earned and is payable in instalments or lump sum and is taxed at a flat rate of 2%. In addition to the salaries quoted above, the main benefits include: support for approved research; rent-free accommodation; appointment and repatriation airfares for appointee and dependants; financial assistance towards the cost of transporting personal effects to and from Papua New Guinea; 6 weeks annual recreation leave with home airfares available after each 18 months of continuous service; generous education subsidies for children attending schools in PNG and overseas; a salary continuation scheme to cover extended illness or disability. The terms and conditions are under review.

Applications will be treated as strictly confidential and should include a full curriculum vitae, a recent photograph and the names and addresses of three referees. In order to expedite the appointment procedure, applicants are advised to ask their referees to send confidential reports directly to the University without waiting to be contacted. Applications should be forwarded to the Deputy Registrar (Staffing), University of Papua New Guinea, PO Box 320, University Post Office, Papua New Guinea. Applications close on 31 August 1985. Candidates in UK should also send a copy of their application to the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. (W1987)A

THE ANIMAL AND GRASSLAND RESEARCH INSTITUTE A BIOCHEMIST

Is required at Shinfield, Reading, to join a team investigating the hormonal control of intermediary metabolism and nutrient partitioning in ruminants. Current interest is focussed on the use of naturally occurring and modified forms of bovine growth hormone to stimulate milk yield in dairy cows and body growth in lambs. Facilities are available for whole animal studies using isotope dilution techniques, colorimetry, metabolite analysis and radioimmunoassay and also for in vitro tissue culture and bioassay.

A suitable candidate will have a First or Upper Second Honours Degree in Biochemistry, with at least four years relevant postgraduate experience and preferably a PhD. Candidates with interests in animal production and/or endocrinology will be preferred.

The appointment will be on the Senior Scientific Officer scale £9,772 to £12,653.

Application forms from the Personnel Officer, Animal & Grassland Research Institute, Hurley, Maidenhead, Berks, SL6 5LR, to be returned by 18 July 1985 quoting S/D/49. (7040)A

UNIVERSITY OF CAMBRIDGE UNIVERSITY LECTURERS AND UNIVERSITY DEMONSTRATORS

in the Department of Anatomy
(four posts)

Applications are invited for four posts of University Lecturer or University Demonstrator (the equivalent of University Assistant Lecturer); not more than two of these posts will be filled at the Lecturer level. Appointment will commence on 1 December 1985, or as soon thereafter as possible. Candidates should be graduates in Medicine or Science and will be required to teach and to contribute to research, for which there are excellent facilities. The teaching and research of the department covers a wide field including topographical and functional anatomy, neuroscience, developmental biology and cell biology. The Department seeks a person with a particular interest in developmental or cell biology to fill one post.

Stipends: University Lecturer £10,330 (by eleven increments) to £15,930. University Demonstrator, £7,980 (by four increments) to £9,860.

Further information about the duties and conditions of appointment may be obtained from the Secretary of the Appointments Committee for the Faculty of Biology 'B', 19 Trumpington Street, Cambridge, CB2 1QA, to whom applications (10 copies) including a curriculum vitae, list of publications and the names of not more than three referees, should be sent so as to reach him not later than 13 September 1985. (6997)A

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY CENTRE FOR BIOTECHNOLOGY

Research Staff

A new building has been provided for the Centre for Biotechnology at Imperial College, which links the biochemistry and chemistry departments, and which is immediately adjacent to the Fermentation Plant of Imperial Biotechnology Ltd. The Centre is now able to recruit staff in support of work in the general area of Chemical Transformations by Micro-Organisms. Applications are invited for the following posts:

Research Officer in Microbial Chemistry (Ref: ROMC)

This is an SERC funded post-doctoral position available in the first instance for two years, to continue an NMR study of the microbial transformations of aromatic compounds. Preference will be given to applicants with a degree in chemistry, experience in NMR experiments and the interest to monitor in vivo metabolic and physiological events: It is anticipated that H, C, F and P will be the principal nuclei monitored. The work envisaged is in collaboration with Professor D.W. Ribbons and Dr A.E.G. Cass. This position is available from October 1985, but a later starting date will be considered for well-qualified and motivated individuals. Salary in the academic-related scale 1A £8,753 — £13,383 including London Allowance + USS.

Serc Studentship (Ref: SQUO)

SERC have provided a quota studentship to the Centre for Biotechnology at Imperial College for research leading to a Ph.D. degree. Applications are invited from persons with honours degrees (at least Upper Seconds) in chemistry, biochemistry, microbiology or related subjects, to initiate a programme of research on the Microbial Transformations of Alkenes with a view to the Generation of Chiral Intermediates for Chemical Synthesis, and a fundamental study of Enzymic Oxygenative Processes. The work will be supervised by Dr D.J. Leak.

SERC Studentship (Ref: SB10)

A Studentship provided by the Biotechnology Directorate of SERC is available for a research project leading to a Ph.D. degree on the generation of novel microbial metabolites which are seen as useful intermediates in chemical synthesis of polymers, fused ring systems and complex natural products. The work is to be supervised by Professor D.W. Ribbons, Dr D.J. Leak (Biotechnology) and Professor S.V. Ley (Organic Chemistry). Candidates should have at least an Upper Second Class honours degree in Chemistry (preferable) or Biochemistry or Microbiology.

Research Studentship (Ref: Biolum)

An industrially funded Research Studentship is available to work in the Biosensors Group at the Centre. The project involves the development of bioluminescence systems for ultra-trace analysis; this work is being carried out in collaboration with the Department of Electrical Engineering. Candidates with an honours degree in either chemistry or biochemistry are invited to apply.

Applications should be sent to Professor D.W. Ribbons, Centre for Biotechnology, Imperial College of Science and Technology, South Kensington, London SW7 2AZ by July 15, 1985, and these should include a curriculum vitae and the names of three referees. (7046)A

ASSISTANT OR ASSOCIATE PROFESSOR (Molecular and Cell Biology)

The Biology Programs of The University of Texas at Dallas invite applications for two tenure-track positions, one at the Assistant Professor level and one at the Assistant or Associate Professor level, beginning 1 September 1986. While the area of specialization is open, applicants should be actively engaged in research in the broad area of molecular or cellular biology, have at least two years postdoctoral experience and show evidence of independent, productive work sufficient to compete for federal grant support. The University has a small (60 majors) upper-level undergraduate biology program and a graduate (60 M.S./Ph.D.) biology program, with extensive extramural supported research in molecular and cellular biology. Successful candidates will be expected to participate in teaching of molecular biology (in biochemistry/biophysics OR molecular genetics), supervise graduate student research, develop a vigorous and independent research program and interact with the current faculty.

Send a curriculum vitae (indication of sex and ethnicity for Affirmative Action statistical purposes is requested but not required), a short description of research plans and names of three or more references to:

Academic Search #271
The University of Texas at Dallas
P.O. Box 830688
Richardson, Texas 75083-0688

Applications should be received by October 15, 1985.

The University of Texas at Dallas is an Affirmative Action/Equal Opportunity Employer. (NW2)A



LOUGHBOROUGH
UNIVERSITY
OF TECHNOLOGY

RESEARCH POSTS IN CHEMICAL ENGINEERING

Several opportunities are available at various levels for work on the following topics.

1. Post-Doctoral

"Characterisation of novel commercial semi-porous membranes for reverse osmosis and ultrafiltration".

2. Research Assistants

- (a) "Hazardous zone definitions in plant layout"
- (b) "Solids control in drilling muds used in oil field operations"
- (c) "On-line monitoring of particulates in fluids used for semi-conductor manufacture"
- (d) "Design of process systems to produce ultra clean fluids for use in semiconductor production"

3. Research Studentships

- (a) "Process Computer Alarm Systems" funded by SERC-BP.
- (b) An SERC studentship is also available for a candidate to work on a mutually agreed topic.

Candidates for Post 1 should have a Ph.D. Starting salary will be not less than £7,520.

For posts 2 and 3 candidates should have a good honours degree in chemical engineering or a related discipline and can register for a higher degree.

Salaries for posts 2, not less than £6,600. Studentship grant is currently £2,585 minimum plus fees.

Write or telephone Dr. A.S. Ward, Department of Chemical Engineering, University of Technology, Loughborough, Leics. LE11 3TU. Tel: 0509 263171 extn. 5033. (7007)A

L'UNIVERSITE DE LAUSANNE

ouvre une inscription en vue de pourvoir, dès le 1er septembre 1986, un poste de

PROFESSOR ASSOCIE

rattaché à l'Institut de Zoologie et d'Ecologie animale (IZEA) de la Faculté des sciences.

Il s'agit d'un poste à plein temps, comportant de l'enseignement (écologie quantitative, génétique des populations et spéciations) et de la recherche (relations phylétiques et écologie des mammifères).

Les candidatures (curriculum vitae, liste des publications, tirés à part des publications les plus significatives) doivent parvenir avant le 30 septembre 1985 au professeur Bernard TESTA, doyen de la Faculté des sciences, Collège propédeutique, 1015 Lausanne-Suisse. (W1993)A

CHAIRMAN — DEPARTMENT OF PHYSIOLOGY

University of Colorado Health Sciences Center seeks a chairperson for the Department of Physiology. Those interested should contact: John V. Weil, M.D., Chairman, Physiology Search Committee, University of Colorado Health Sciences Center, 4200 E. Ninth Ave., Campus Box B-133, Denver, CO 80262. AA/EEO. (NW8)A

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE (University of London)

DEPARTMENT OF BIOCHEMISTRY AND CHEMISTRY RESEARCH ASSISTANT

to work on biophysical and biochemical studies of the cardiac sarcoplasmic reticulum. Applicants should have a PhD in biochemistry and preferably experience in either working with biological membranes or in the area of phosphorylation-mediated control of enzyme activity. Experience in the use of physical techniques in biology an advantage but not essential.

The post is for approximately 2 years, supported by a grant from the British Heart Foundation to Professor D Chapman.

Full curriculum vitae and the names and addresses of two referees should be sent to School Office, RFHSM, Rowland Hill Street, London NW3 2PF (794-0500 Extn 4262). Please quote reference DC/RA. Closing date 19 July 1985. (7033)A

PUBLIC HEALTH LABORATORY SERVICE BOARD

PHLS Centre for Applied
Microbiology & Research

THERAPEUTIC PRODUCTS
LABORATORY

PROCESS DEVELOPMENT BIOCHEMIST

A protein chemist/enzymologist is required for the development of purification processes and characterisation of new therapeutic products from human pituitary glands and recombinant DNA technology using pharmaceutically acceptable techniques. The work will include research into methods of protein immobilisation for immunoaffinity chromatography and the development of tests to assess the safety of parenteral products derived from cloned or fused cell lines.

Applicants should possess a good Honours Degree in Biochemistry/Chemistry and at least three years relevant post graduate experience. Further information may be obtained from Dr G W Jack, Head of Process Development Section, TPL.

The successful applicant will be appointed as a Basic Grade Microbiologist.

Salary will be in the range £6,559 — £8,725.

National Health Service terms and conditions will apply.

Applications including curriculum vitae and the names and addresses of three referees should be sent to the Personnel Officer, PHLS Centre for Applied Microbiology & Research, Porton Down, Salisbury, Wiltshire SP4 0JG.

The closing date for applications is 29 July 1985.

Please quote Reference 29 when applying. (6995)A

THE UNIVERSITY OF THE SOUTH PACIFIC Suva, Fiji

Applications are invited for the following position:

LECTURER I/ SENIOR LECTURER IN CHEMISTRY — POST 85/37

Applicants should have a proven record of teaching and research in analytical, inorganic or physical chemistry and some experience in administration will be an advantage. Competence at teaching all the main areas of analytical, inorganic or physical chemistry to general degree level will be required. Staff are expected to supervise postgraduate students for higher degrees and may be involved in teaching courses through extension and in consultancy projects for associated University Institutes. The successful applicant is expected to take up the position on January 1, 1986 or as soon as possible after that date. Appointment will be for a contract period of three years and may be renewable by mutual agreement.

Salary scales: Lecturer I F\$13,921 — 18,919, Senior Lecturer F\$19,525 — 22,815 (£1 = F\$1.49 on 11-6-85). Salary will be in accordance with qualifications and experience.

In addition, the University provides gratuity amounting to 15% of basic salary, appointment allowance and, subject to the University's current housing policy, partly furnished accommodation at a rental of 12½% of salary. The University will contribute a sum equivalent to 10% of the appointee's salary towards his superannuation obligations. Candidates should send three copies of their curriculum vitae with full personal particulars, names and addresses of three referees and date of availability, to the Registrar, University of the South Pacific, PO Box 1168, Suva, Fiji, to reach him no later than 3 August 1985.

Further information on request from the Registrar or from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, to whom candidates in-UK should also send an additional copy of their applications. (W1997)A

POSTDOCTORAL POSITION

Position available to study the biochemistry, immunology and genetics of a human malaria parasite. Duration — two years — starting as soon as possible. Experience in biochemistry, immunochemistry or recombinant DNA techniques desirable.

Send CV and the names of three referees to: Dr Joseph Inselburg, Department of Microbiology, Dartmouth Medical School, Hanover, NH 03756, (603) 646-7666.

Dartmouth College is an affirmative action employer. (NW9)A

**THE ANIMAL VIRUS
RESEARCH INSTITUTE**
Ash Road, Pirbright, Woking,
Surrey GU24 0NF
**HIGHER SCIENTIFIC
OFFICER**

Following the restructuring of the Animal Virus Research Institute, applications are invited for a new post of Higher Scientific Officer to work in the Division of Molecular Biology, Director's Group. The appointment to commence as soon as possible. The successful candidate will work on the mechanisms of replication of fowl plague virus RNA.

Candidates should have a First or Upper Second Class Honours degree with at least two years post graduate qualifying relevant research experience.

Salary scale £7,788 — £10,541, sick leave and non-contributory superannuation schemes, 22 days annual holiday. House may be available for suitable married applicant.

Applications to include a curriculum vitae and the names of two referees should be sent as soon as possible to: The Secretary, The Animal Virus Research Institute, Ash Road, Pirbright, Woking, Surrey GU24 0NF. Tel: 0483 232441/8.

The Animals Virus Research Institute is an Equal Opportunities Employer. (6945)A

UNIVERSITY OF DUNDEE
DEPARTMENT OF BIOCHEMISTRY
**POST-DOCTORAL
RESEARCH ASSISTANT**

Applications are invited for the above SERC-funded position which forms part of a small group studying the mechanism of cofactor acquisition by molybdoenzymes in *E. coli*. The project involves the biochemical analysis of a series of mutants which are defective in molybdoenzyme biosynthesis. Enzymological and molecular genetic approaches are being employed. Persons with experience in either or both of these areas are preferred but other strong applicants will also be considered. Further information may be obtained from Dr D H Boxer (0382 23181, ext 4250). The position is available for up to three years starting on 1 October 1985.

Appointment will be on the Research & Analogous IA scale currently £7,520 — £12,150 depending on qualifications and experience.

RESEARCH STUDENTSHIP

A SERC Studentship awarded specifically for the above project is also available. The project could either centre on the molecular genetic or enzymological aspects of the work. Applicants for this position should apply as below but directly to Dr Boxer.

Please apply enclosing a curriculum vitae and the names of two referees to The Personnel Officer, The University, Dundee, DD1 4HN by 25th July 1985. Please quote REF EST/75/85J. (7000)A

**UNIVERSITY OF
AUCKLAND**
New Zealand
**LECTURESHIP IN
GEOCHEMISTRY**
(3 year term)

Closing date: 17 August 1985

Applicants should have advanced qualifications and have teaching and research experience and interests in fluid geochemistry and physical chemistry, and preferably have experience with the geochemistry of geothermal fluids. The successful applicant will be required to teach, supervise laboratories and conduct research within the Geothermal Institute and Department of Geology.

Commencing salaries will be established within the appropriate scale: Lecturers NZ\$23,622 — \$27,928, Senior Lecturers NZ\$29,430 — \$37,240.

Conditions of Appointment and Method of Application are available from the Assistant Registrar (Academic Appointments), University of Auckland, Private Bag, Auckland, New Zealand, or the Secretary General, Association of Commonwealth Universities (Appts), 26 Gordon Square, London WC1H 0PF. Applications, in accordance with the Method of Application should be forwarded as soon as possible, but not later than the closing dates stated. (W1998)A

**THE INSTITUTE OF
CANCER RESEARCH:**
**TECHNICIAN/RESEARCH
OFFICER**

is required to continue an established project funded by the CRC/MRC at the Institute's laboratories at Sutton, Surrey. The project involves the use of mouse aggregation chimaeras. These are produced by combining embryos from two different mouse strains: the tissues of resulting progeny are a mosaic of cells of each parental type, which can be stained immunohistochemically. The mosaicism is being used to examine clonal organisation and interaction in normal and neoplastic tissue. Training is available in a wide range of techniques relevant to the project: but experience of histology would be an advantage. Post available now: starting date to be arranged.

Salary in the range of £5,738 — £8,492 + £623 pa. London Weighting allowance.

Applicants are advised that in the majority of the Institute's premises smoking is prohibited.

Prospective applicants are encouraged to telephone Dr Bruce Ponder on 01-643 8901 Ext 293 for further details.

Applications in duplicate with the names and addresses of two referees should be sent to the Personnel Officer, Institute of Cancer Research, 17A Onslow Gardens, London SW7 3AL, quoting reference 301/B/83. (7008)A

Marine Laboratory, Aberdeen
**Department of Agriculture
and Fisheries for Scotland**

Chemist

To join a group investigating the sources, distribution, fate and controlling processes of trace metals in the marine environment. You will participate in laboratory and field investigations including research cruises and you will be expected to adopt a multi-disciplinary approach to these studies and to be capable of innovative and unsupervised work.

You should have a degree, HND, HNC or equivalent in chemistry, chemical oceanography or other relevant scientific subject. Postgraduate experience in marine chemistry or chemical oceanography would be advantageous.

Appointment will be as Scientific Officer £6190-£8560 with starting salary according to qualifications and experience. Ref: SA/35/DLC.

Physical Oceanographer

To join a group involved in oceanographic research programmes related to environmental problems of fishery management and the impact of pollution on marine life.

You should have a 1st or 2nd class honours degree in physics or a relevant subject with at least 2 years' postgraduate experience in physical oceanography. A knowledge of the skills needed to make full use of oceanographic instrumentation and computer facilities would be an advantage.

Appointment will be as Higher Scientific Officer £7785-£10,540 or Senior Scientific Officer £9770-£12,650 with starting salary according to qualifications and experience. Ref: SA/36/DLC.

For further details and an application form (to be returned by 26 July 1985) write to Scottish Office, Personnel Division, Room 208, 16 Waterloo Place, Edinburgh EH1 3DN.

Please quote appropriate reference.

The Civil Service is an equal opportunity employer

(7044)A

**Scientific
Civil Service**

**CROP PHYSIOLOGIST/
POMOLOGIST**

The Department of Pomology, University of California, Davis, invites applications to fill a tenure track position as Assistant Professor and Assistant Pomologist (10% teaching/90% research). The appointee will be expected to conduct research related to soil-plant-water relationships and irrigation strategies for deciduous fruit and nut crops. Potential research projects include, but are not limited to, the effects of soil moisture and plant water status on tree growth, root behaviour, crop yield and quality, and predisposition of deciduous fruit trees to pests and diseases. Interaction with Cooperative Extension personnel and participation in student advising and upper division and/or graduate pomology courses will be expected. A Ph.D. degree in plant physiology, pomology, botany, or a related field is required. Previous experience with tree crops is desirable but not prerequisite.

Submit curriculum vitae, official undergraduate and graduate transcripts, publications list, reprints, statement of research and teaching interests, and names and addresses of at least three referees to: Ted M. DeJong, Search Committee Chairperson, Department of Pomology, University of California, Davis, CA 95616 prior to October 1, 1985.

The position will be available for appointment December 1, 1985, or later depending on availability of the successful applicant.

In conformance with application law and University policy, the University of California is an Affirmative Action/Equal Opportunity Employer. (NW6)A

CARCINOGENESIS

The University of Texas System Cancer Center (UTSCC)

Science Park-Research Division

Applications are invited for an assistant professor level faculty position in a multidisciplinary research laboratory studying cellular and molecular mechanisms of carcinogenesis. Research programs at Science Park, located in Buescher State Park 40 miles east of Austin, include somatic cell genetics, DNA repair and mutagenesis, carcinogen metabolism and adduct formation, tumor promotion, mechanisms of lymphomagenesis, normal and tumor cell differentiation, biochemical genetics, and growth factors. Candidates should have an independent research program that would interface with one or more of the above areas as well as a record of peer-reviewed grants and research publications. Preference will be given to individuals who have an active program on the cellular and molecular mechanism(s) by which dietary fat influences the induction of cancer in the mammary gland, large bowel, or pancreas. The Science Park provides competitive salary and benefits, a stimulating research environment, postdoctoral and graduate students, and opportunities for interaction with faculty at nearby institutions, including the University of Texas Health Science Center in Houston, University of Texas at Austin, and Texas A&M University.

Interested applicants should send complete curriculum vitae, the names of three referees, and a brief statement of research interests to: Dr Thomas J. Slaga, Director, UTSCC, Science Park-Research Division, P.O. Box 389, Smithville, Tex. 78957; Telephone: 512-237-2483.

An Equal Opportunity/Affirmative Action Employer.

(NW5)A

LIFE SCIENCE RESEARCH ASSISTANT: REQUIRE Ms IN CHEMISTRY

1-3 years experience in separation of macromolecules using gel chromatography and HPLC. Ability to set up HPLC and interface the instrument with computer required. Experience in microanalysis by immunodiffusion and knowledge of osmometer, creatinine analyzer, autoanalyzer, oncometer and fractionator necessary. Knowledge of polymer and protein chemistry desirable. 1844-2331/month.

Closing date August 4, 1985. Send résumé to: Carol Young, S215, Stanford Medical Center, Stanford, CA 94305.

(NW4)A

RESEARCH TRAINING IN SALIVARY SECRETION

Positions available starting summer 1985 at the University of Washington for research training in the biochemistry, cell biology, microbiology, physiology, pharmacology and/or pathology of salivary secretion. Applicants should have Ph.D. or professional degree (M.D., D.D.S., D.V.M., or equivalent) and must be U.S. citizens or have permanent visas.

Graduate programs leading to Ph.D. or M.S. are available. Stipends: \$15,996 to \$30,000, depending upon exercise.

Send curriculum vitae, three references and statement of research interests to: Dr Kenneth Isutsu, Department of Oral Biology, SB-22, University of Washington, Seattle, Wash. 98195 at earliest possible date.

An Affirmative Action Equal Opportunity Employer.

(NW7)A

UNIVERSITY OF ST ANDREWS MRC Cognitive Neuroscience Research Group POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for the above post to work on an MRC funded project investigating the behavioural and neurological sequelae of closed head injury. The project involves the employment of psychometric and neuropsychological testing procedures, and the collection of scalp-recorded evoked potentials in a range of paradigms. Applicants should be familiar with at least some of these techniques, and should possess a valid driving licence.

The appointment will be for 31 months, starting salary near the bottom of the 1A scale (1st point £7,520). Further details of the post are available from Dr M D Rugg, Department of Psychology, University of St Andrews. Applications (two copies preferably in typescript) with the names of two referees should be sent to the Establishments Officer, The University, College Gate, St Andrews, Fife, KY16 9AJ, to arrive not later than 24 July 1985.

(7028)A

THE UNIVERSITY OF WALES ABERYSTWYTH DEPARTMENT OF AGRICULTURE RESEARCH OFFICERS

Applications are invited for two MAFF funded posts tenable for a period of three years commencing 1 August 1985, or as soon as possible thereafter, to investigate the Nitrogen Cycle on Organic Farms.

One post will concentrate on monitoring the Nitrogen cycle on a whole farm and individual field basis and the other on studying the availability of Nitrogen from the soil. Salary within the range £6,600 to £7,520 per annum.

Application forms and further particulars can be obtained from the Staffing Officer, The University College of Wales, Old College, King Street, Abersystwyth, SY23 2AX (Tel 0970 3177, Ext 207). Closing date for applications: Friday, 19 July 1985.

(7050)A

UNIVERSITY OF AUCKLAND New Zealand CHAIR IN NEUROPHYSIOLOGY (DEPARTMENT OF PHYSIOLOGY — SCHOOL OF MEDICINE) CLOSING DATE: 13 SEPTEMBER 1985

This is a newly established Chair open to both medically and non-medically qualified applicants. Medical qualifications must be registrable in New Zealand. The Chair has been established with a view to promoting interdisciplinary graduate studies in the neurosciences. Applicants should have postgraduate qualifications in an appropriate field and an established record of research achievement in neurophysiology. Teaching experience at both undergraduate and graduate levels is essential, and the successful applicant will be expected to promote the development of a Centre for Graduate Studies in Neural Sciences. Commencing salaries will be established within the ranges NZ\$58,174 — \$64,121 per annum for medically qualified, or NZ\$45,319 — \$56,602 for non-medically qualified applicants, having regard to the qualifications of the candidates concerned.

CHAIR IN RADIOLOGY (DEPARTMENT OF ANATOMY — SCHOOL OF MEDICINE) CLOSING DATE: 20 SEPTEMBER 1985

Applications are invited from persons with medical qualifications registrable in New Zealand, and with a higher postgraduate qualification in Radiology. The successful applicant will be expected to organise and assist with both undergraduate and postgraduate teaching in radiology in the School of Medicine and Auckland Hospital, to control the experimental radiological laboratory within the School of Medicine and to engage in, encourage and supervise research. Commencing salaries will be established within the range NZ\$58,174 — \$64,121 per annum, having regard to the qualifications of the candidates concerned.

Conditions of Appointment and Method of Application are available from the Assistant Registrar (Academic Appointments), University of Auckland, Private Bag, New Zealand, or from the Secretary General, Association of Commonwealth Universities (appts), 36 Gordon Square, London WC1H 0PF. Applications, in accordance with the Method of Application, should be forwarded as soon as possible but not later than the closing dates states.

(W1986)A

CSIRO

AUSTRALIA RESEARCH LEADER (IMMUNOLOGY) \$39,692 — \$52,212 AUSTRALIAN NATIONAL ANIMAL HEALTH LABORATORY GEELONG VIC

The Australian National Animal Health Laboratory (ANAH) is a high security microbiological laboratory for the study of animal diseases exotic to Australia. It is located at Geelong, a city with a population of 170,000 situated 80 kms south of Melbourne. When fully operational in 1985/86 it will employ a staff of 178, including 30 scientists.

ANAH has a most important role in the diagnosis and control of exotic diseases of livestock in Australia. To carry out its role it undertakes disease diagnosis, research, development and testing of vaccines, and training of field and laboratory personnel. These functions support and complement those undertaken by the various disease control authorities in Australia.

Currently there are four research groups in ANAH — Epidemiology, Molecular Virology, Pathogenesis and Immunity, and Microbiological Security with expertise in microbiology, immunology, veterinary pathology and molecular biology. Facilities are available within the Laboratory for gene cloning and sequencing, protein synthesis and sequencing, electron microscopy, histopathology and immunology. The Laboratory is currently seeking to appoint a co-ordinator for its Pathogenesis and Immunity Group. The appointee will establish the group and lead research into the pathogenesis and immunity of virus diseases of livestock.

Applications are invited from persons with a PhD or equivalent degree in a relevant field, plus a record of extensive research achievement in the field of viral immunology. Evidence of scientific leadership would be an advantage. Appointment to CSIRO is for an indefinite period and carries Australian Government superannuation benefits, subject to normal conditions. The position of Group Co-ordinator however will be offered for a negotiable term, in the order of three to five years, with subsequent options for a further term if mutually desired.

Enquiries or applications stating relevant personal and professional details, the names of at least three referees, and quoting reference Nc A6877 should be directed to: Mr V. A. Snowdon, Chief, CSIRO Australian National Animal Health Laboratory, PO Bag 24, GEELONG VIC 3220, 26 July 1985.

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER. (W2000)A

Please mention
nature
when replying to these advertisements

**THE UNIVERSITY
COLLEGE OF WALES
ABERYSTWYTH**

DEPARTMENT OF BOTANY AND
MICROBIOLOGY
**POST-DOCTORAL
RESEARCH ASSOCIATE**

Applications are invited from Plant Physiologists, Plant Biochemists or Natural Products Chemists, to join a research group supported by an AFRC special block grant working on the biochemistry of endogenous plant growth regulators. The current research interests of the group include the biosynthesis and metabolism of cytokinins and the pathway and control of abscisic acid biosynthesis in plants. The appointment will be for an initial period of 18 months within the salary range £7,520 to £8,450 per annum.

Application forms and further particulars can be obtained from the Staffing Officer, The University College of Wales, Old College, King Street, Aberystwyth, SY23 2AX (Tel 0970 3177, Ext 207). Informal enquiries can be made to Dr Roger Horgan (Tel 0970 3111, Ext 3054). Closing date for applications: Friday, 26 July 1985. (7051)A

**THE UNITED MEDICAL
AND DENTAL SCHOOLS**

St Thomas's Campus

RESEARCH ASSISTANT
(possibly post-doctoral)

required to work on immunity to and the molecular biology of rubella virus. Virology experience essential; some knowledge of ELISA and PAGE techniques and use of monoclonal antibodies an advantage.

Funding for post is currently being sought; anticipated start date early September.

Salary according to age and experience.

Applications with cv and names and addresses of two referees to Staffing Officer, Dean's Office, St Thomas's Hospital Medical School, London SE1 7EH, not later than 18th July, 1985 quoting ref: STH/V/38. (6859)A

**BEN GURION UNIVERSITY
OF THE NEGEV
BEER SHEVA — ISRAEL**

DEPARTMENT OF BIOLOGY

Two tenure-track academic positions are available in the Department of Biology, for scientists working at the molecular or organismic level in modern fields of biology. Teaching responsibilities include undergraduate and graduate courses. The level of appointment is dependent upon the academic achievements and experience of the candidate.

Applications including CV, list of publications, description of areas of research interests and names and addresses of at least three referees should be sent to Prof Chanan Itai, Chairman, Department of Biology, Ben Gurion University of the Negev, Beer Sheva, Israel. (W1988)A

**UNIVERSITY OF DUNDEE
DEPARTMENT OF BIOCHEMISTRY
ORGANELLE DIVISION
DURING MITOSIS**

A post-doctoral research assistant is required to work on the division of the Golgi complex during mitosis in animal cells. The aim is to understand the molecular basis of this process. Preference will be given to candidates with training in membrane biochemistry and/or enzymology.

This project is funded by the CRC and the position is for three years commencing 1 October 1985. Salary will be in the range £7,520 — £12,150 per annum.

Applications, including a curriculum vitae, research summary and the names and addresses of three referees, should be sent to the Personnel Officer, University of Dundee, Dundee DD1 4HN, Scotland as soon as possible. Informal enquiries concerning this project should be addressed to Professor Graham Warren, who is presently at the European Molecular Biology Laboratory, Postfach 10.2209, 6900 Heidelberg, West Germany, telephone number 010 49 6221 387 324. Please quote reference EST/74/85(J). (6999)A

**THE ANIMAL
HEALTH TRUST**

offer a

**PACKET BOAT
MEMORIAL
FELLOWSHIP**

In the Physiology Unit

**CARDIORESPIRATORY
PHYSIOLOGIST**

As part of an integrated approach to the physiological and biochemical response of the racehorse to exercise, an experienced cardiorespiratory physiologist is required to join a team of veterinarians and biochemists. The response of animals exercised on the track, as well as in a newly built Exercise Unit containing a high speed treadmill and other facilities will be investigated. Emphasis will be on pulmonary function and gas exchange, with studies to be undertaken in both normal horses and those with respiratory disorders. Some comparative studies in other species will be undertaken. The work will also be part of a collaborative project with the Department of Zoology and Comparative Physiology, University of Birmingham.

The Fellowship will be for 4 years. Salary range £8,920 — £10,720.

For further details contact Dr D H Snow. Application forms available from the Administration Secretary, to be returned to The Director, The Animal Health Trust, Balaton Lodge, Snailwell Road, Newmarket, Suffolk CB8 7DW (Tel: 0638-661111). Closing date: 16th August 1985. (7053)A

CSIRO

AUSTRALIA

RESEARCH FELLOW

\$26,001 — \$38,243

DIVISION OF SOILS

ADELAIDE SA

FIELD: Plant physiology/biophysics.

GENERAL: The CSIRO Division of Soils conducts research into most aspects of soil science, including the physics, chemistry and biology of soils, and the integrative disciplines of pedology and geomorphology. It also seeks the application of its research in agriculture and other areas of science and technology. The Division has laboratories in Adelaide, Brisbane, Canberra and Townsville.

DUTIES: The appointee will undertake research on the movement of water both to and from plants. Initially, the research will evaluate the usefulness of the natural isotopes deuterium and oxygen-18 to elucidate details of the mechanism of water loss from plants.

The appointee will be based at the Division's Adelaide Laboratory which is equipped with a VG602 mass spectrometer and extensive growth cabinet and glasshouse facilities.

QUALIFICATIONS: Applicants should have a PhD degree or equivalent qualifications with training in some aspects of plant science. Some knowledge of physics would be an advantage.

TENURE: A term of 3 years. Australian Government superannuation benefits are available.

APPLICATIONS: Stating relevant personal and professional details, the names of at least two scientific referees and quoting reference No A0997 should be directed to:

The Chief
CSIRO Division of Soils
Private Bag No 2
GLEN OSMOND SA 5064

by 26 July 1985
CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER
(W2006)A

UNIVERSITY OF SURREY

DEPARTMENT OF CHEMISTRY

Applications are invited from Chemistry graduates, or those about to graduate, for the following research studentships which will become available as from 1st October, 1985.

1) A CASE studentship in conjunction with ICI Pharmaceuticals to study

A Thermodynamically-Based
Scale of Hydrogen Bond
Basicities

(Dr M H Abraham)

2) A CASE studentship in conjunction with the Imperial Cancer Research Fund to study

Mechanisms of Aromatic
Hydroxylations

(Dr J R Jones)

3) A CASE studentship in conjunction with ICI to study

The Synthesis of Polysulphones
from Arylsulphonic
Acids and PC1 25 3

(Professor J B Rose)

Interested persons should send their curriculum vitae and the names and addresses of two referees to Dr J R Jones, Chemistry Department, University of Surrey, Guildford, GU2 5XH, as soon as possible. (7037)A

**AGRONOMIST
Bedfordshire**

Our Client is an international leader in plant breeding and seed marketing. An Agronomist is required to set up and develop field trials in the U.K.

Applicants must have 1 or 11 Hons. in Agricultural Sciences or Botany with Agriculture. Relevant experience would be beneficial. Essential is a long term commitment to agronomy, an understanding of farming requirements and the ability to record and interpret trial data. A practical approach together with physical fitness is even more important since considerable planting of crops is involved in several locations.

This is an exceptional career opportunity with management potential for those with the appropriate commitment to commercial seed development.

Attractive salary, according to experience. Starting date: August/September 1985. A driving licence is essential.

Contact R.G. Bubb, Director with C.V. or send for application form and further details to DSA Appointments, Flitch House, 42 High Street, Great Dunmow, Essex. CA6 1AH. Telephone (0371) 5106. (7020)A



HIGH MODULAS POLYMERS

ULIS (University of Leeds Industrial Services) Ltd., wishes to make contact with an experienced

RESEARCH WORKER

to develop applications for high modulus polymers, a field in which there is a very active programme of research at Leeds University. The post, which is for two years in the first instance, could be filled by a graduate/post graduate in physics, chemistry engineering or materials science

Salary according to age and qualifications in the range £6,600 — £8,920/£7,250 — £12,150.

Please send C.V. and full details with 2 referees to M.D., ULIS Ltd. 175 Woodhouse Lane, Leeds 2, marking envelope "Confidential". (7017)A

UNIVERSITY OF WISCONSIN-MADISON Mycology/Microbial Ecology RESEARCH ASSOCIATE (postdoctoral)

position available 1 June 1985 on the BIOTECHNOLOGY and microbial ecology of fungal pathogens (biocontrols) to watermillfoil. Research aspects include mass culture and strain improvement techniques, interaction with other control measures, and tracking marked populations in water and sediment. Funding for two years. Salary competitive and commensurate with qualifications.

Send résumé with names of 3 referees to: Dr. John H. Andrews, Department of Plant Pathology, 1630 Linden Drive, Madison, WI 53706.

An affirmative action/equal opportunity employer. (NW1914)A

POSTDOCTORAL FELLOW

The Center for Separation Science, College of Engineering, and the Department of Pharmacology, College of Medicine, have a joint full-time position available to study neuropeptide processing (metabolism) in animal tissues. The focus of the studies is applying state-of-the-art separation techniques to the isolation and purification of peptide specific proteolytic enzymes altered by disease or drugs. A strong background in biochemistry and pharmacology desired.

Applications will be accepted until August 15, 1985.

Salary: £18,000.

Send résumé, copies of representative publications, and names of three referees to: Dr. Thomas P. Davis, Department of Pharmacology, University of Arizona, College of Medicine, Tucson, Arizona 85724.

Equal Opportunity/Affirmative Action Employer. (NW3)A

HOUGHTON POULTRY RESEARCH STATION Grant-Aided by the Agricultural & Food Research Council TEMPORARY MOLECULAR BIOLOGIST

Applications are invited for the above post to work on molecular aspects of pathogenesis of Marek's disease. Areas of interest are in situ hybridization and characterization of RNA transcripts and their translated products in infected cells.

Candidates should have recently completed a PhD degree or be graduates with experience in molecular biology of nucleic acids. A background in virology would be an advantage but is not essential.

The post is for a period of 2 years and is supported by the Leukaemia Research Fund. Informal enquiries to Dr N Ross (Tel 0480-64101).

The appointment will be made to the Higher Scientific Officer grade (salary £7,788 × 6 — £10,541) or the Senior Scientific Officer grade (salary £9,772 × 7 — £12,653) depending on experience and qualification.

An application form and further particulars may be obtained from the Secretary, Houghton Poultry Research Station, Houghton, Huntingdon, Cambs. The closing date for applications is 25th July 1985. (6972)A

PUBLIC HEALTH LABORATORY SERVICE BOARD Central Public Health Laboratory PRINCIPAL MICROBIOLOGIST Antibiotic Reference Laboratory Division of Hospital Infection

The post is associated with the establishment of an Antibiotic Reference Laboratory by the PHLS Board. The Principal Microbiologist will be expected to play a large part in developing and expanding the work of the new laboratory; in particular the study of the genetics and mechanisms of antibiotic resistance.

Candidates should have a first degree in Microbiology or a related subject, and post graduate experience is essential.

This is a permanent post, salary on the scale £11,927 to £16,730 plus £648 per annum London Weighting NHS terms and conditions of service will apply.

The department may be visited by arrangement with Dr R C George (Telephone 01-200 4400, extension 4222).

Application forms and job description are available from the Personnel Officer, Public Health Laboratory Service Headquarters, 61 Colindale Avenue, LONDON, NW9 5HT. Closing date for application: 31st July 1985. (6992)A

FOOD RESEARCH INSTITUTE, READING FOOD MICROBIOLOGY DEPARTMENT MICROBIOLOGIST, BIOCHEMIST

or person with similar background required to join research group devoted to developing new and improving existing methods for the detection of microorganisms in foods. This post is specifically concerned with the development of novel electrically based methods, in particular diversion of metabolic electron flow by redox mediators. An interest in bioenergetics, electrochemistry or computing would be advantageous.

The appointment will be as Scientific Officer (£6,190 to £8,561) or Higher Scientific Officer (£7,788 to £10,541). Non-contributory superannuation. Equal opportunities employer.

Qualifications: First or upper second class honours degree in Microbiology, Biochemistry or related subject with at least two years' post-qualifying relevant research experience for appointment at the higher grade.

Apply by letter and curriculum vitae to the Secretary, Food Research Institute, Shinfield, Reading, RG2 9AT.

Please quote ref 85/10. (7042)A

THE UNIVERSITY OF LEEDS

DEPARTMENT OF ANIMAL
PHYSIOLOGY AND NUTRITION

POSTDOCTORAL RESEARCH FELLOW

Applications are invited for the above post available from 1 October 1985 for a fixed term of two years.

Applicants must have or shortly expect to obtain a PhD in neurophysiology or a closely related subject.

The project involves a study of the effects of visceral stimuli, including gastrointestinal hormones, on single unit activity in the vagus and splanchnic nerves.

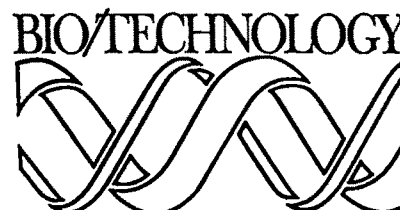
Salary on the IA Range for Research and Analogous Staff (£7,520 — £12,150) (under review), according to age, qualifications and experience.

Informal enquiries may be made to Dr J M Forbes (tel (0532) 431751 ext 7389 or 7385).

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference number 61/20. Closing date for applications 25 July 1985. (7013)A

ATTENTION: R&D MANAGERS, PRINCIPAL
INVESTIGATORS, DEPARTMENT CHAIRPEOPLE,
SENIOR SCIENTISTS

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UNIVERSITY OF NEWCASTLE UPON TYNE

DEPARTMENT OF
RADIOTHERAPY
Cancer Research Unit
JUNIOR RESEARCH
ASSOCIATE

Applications are invited from graduates for a post of Junior Research Associate funded by the North of England Cancer Research Campaign tenable for three years from 1st August 1985. Applicants should hold or expect to receive a good honours degree in a biological science subject. The project will be concerned with the analysis of DNA repair mutants of mammalian cells and the molecular cloning of human DNA repair genes.

Commencing salary will be £6,600 per annum on the range 1B scale.

Applications, together with the names and addresses of three referees should be sent by not later than Thursday, 25th July 1985 to Dr I D Hickson, Cancer Research Unit, University of Newcastle upon Tyne, The Royal Victoria Infirmary, Newcastle upon Tyne, NE1 4LP. (7030)A

UNIVERSITY OF CALIFORNIA

DEPARTMENT OF PHYSICS
ENDOWED CHAIR
PROFESSOR OF PHYSICS

The UCLA Physics Department has an open Endowed Chair position. An appointment will be considered in any field of experimental or theoretical physics. The successful candidate will have a regular University of California tenured faculty position; the endowment income will support the candidates' program.

The successful candidate will be

KINGSTON POLYTECHNIC

Faculty of Science
School of Geological Sciences
LECTURER II/
SENIOR LECTURER IN
GEOLOGY

(Mineral Deposits Geology/Metamorphic and Structural Geology)

Applications are invited for a post of Lecturer II/Senior Lecturer in Geology which is available from 1st September 1985. Applicants should have a high research potential and a strong interest in teaching. Preference will be given to candidates with experience in either mineral deposits geology or metamorphic and structural geology. The successful candidate will be expected to make industrial contacts with a view to developing consultancy.

Salary range L II/SL: — £8,226 — £14,739 including London allowance (under review).

Details and application forms (to be returned by 29th July) from Personnel Officer, Kingston Polytechnic, Penrhyn Road, Kingston upon Thames KT1 2EE. Tel 01-549 1366 ext 287/205. (7019)A

expected to lead an internationally recognized research program, and to teach at the graduate and undergraduate level.

Applicants should send a curriculum vitae, a brief statement of past, present, and future research interests, and the names of 5-10 referees to C Kennel, Chair, Department of Physics, University of California, Los Angeles, CA 90024.

The department is especially interested in identifying women and minority candidates.

The University of California is an affirmative action/equal opportunity employer. (NW1435)A

THE UNIVERSITY OF MANCHESTER POSTDOCTORAL RESEARCH ASSOCIATE

DEPARTMENT OF
CHEMICAL PATHOLOGY

Applications are invited for this post to join a group producing monoclonal antibodies to peptides of clinical importance. Candidates should possess a PhD, have wide experience in methods of peptide chemistry including (immuno) affinity chromatography and preferably monoclonal antibody production and ELISA, and be capable of initiating and expanding research in the agreed field(s) of study on minimal supervision. This is a 2 year appointment starting no later than September 1st, 1985. Salary range pa: £7,520 — £12,150. Superannuation.

Applications, including CV and names, addresses and telephone numbers of 3 referees, should be sent to Dr A. White, Department of Chemical Pathology, Hope Hospital, Salford M6 8HD, from whom further information may be obtained (tel 061-789-7373, extension 212). (6989)A

ROYAL POSTGRADUATE MEDICAL SCHOOL (University of London)

DEPARTMENT OF IMMUNOLOGY
RESEARCH OFFICER

required for a 3 year MRC funded post to assist in a study of the fourth component as a polymorphic marker within the MHC. The work will involve production of monoclonal antibodies and their use in immunoelectrophoresis which will be combined with serological studies of human anti-C4.

Candidates must have a first or good second class honours degree in an appropriate subject.

Salary will be £6,600 plus £1,233 London Allowance, rising to £7,520 plus £1,233 London Allowance in the third year.

Application forms and further particulars from the Personnel Office, Royal Postgraduate Medical School, 150 Ducane Rd, London W12 0HS quoting ref: 21/205/N.

Closing Date: 25 July 1985.

(6994)A

Brighton Polytechnic

DEPARTMENT OF PHARMACY

Applications are invited for the following SERC CASE Awards:

- 1 Diffusion of antibiotic molecules through biopolymer gels, in conjunction with Beecham Pharmaceuticals Research Division;
- 2 Evaluation of the ciliotoxicity of pharmaceuticals, in conjunction with the Wellcome Foundation.

Applicants should possess a good honours degree in Pharmacy, Physiology, Biochemistry or a related discipline. Normal SERC conditions apply and candidates will be expected to register for a higher degree.

Further information and forms of application may be obtained from Personnel Department, Brighton Polytechnic, Mithras House Moulsecoomb, Brighton BN2 4AT. Telephone 0273 693655 Ext. 2536. Closing date July 15, 1985. (7054)A

UNIVERSITY OF BIRMINGHAM

Faculty of Medicine and
Dentistry

DEPARTMENT OF MEDICAL
MICROBIOLOGY
RESEARCH ASSOCIATE

is required to work on molecular biological/immunological aspects of research into a herpes virus vaccine. A background in virology/microbiology/biochemistry or immunology would be beneficial but not essential.

Post available for 12 months from starting date. Salary on scale £6,600 — £8,920 with superannuation.

Applications (3 copies) to Assistant Registrar, Medical School, Birmingham B15 2TJ by 26th July 1985. Quote ref RA/MM/GRS. (7048)A

TWO POSTDOCTORAL POSITIONS

are available immediately for a Ph.D. biochemist and a molecular biologist to participate in studies of gene regulation by steroid receptors. Projects include the purification and characterization of human estrogen and progesterone receptors, cloning of the corresponding genes and preparation of monoclonal antibodies. Experience with steroid receptors, protein purification and analysis, monoclonal antibody production and/or recombinant DNA techniques is desired. Salary is negotiable.

Send curriculum vitae and three letters of references to: Dr Geoffrey L. Greene, The University of Chicago, Ben May Laboratory for Cancer Research, 5841 S. Maryland, Chicago, Illinois 60637. (NW1903)A

BIOTECHNOLOGISTS!

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Houndmills, Basingstoke, Hants.
RG21 2XS, UK

or

Paul Siman, Bio/Technology,
65 Bleecker St., New York, NY 10012.

WORKSHOPS

DNA SEQUENCING WORKSHOP

GIBCO BRL are pleased to announce a DNA sequencing workshop to be held in Glasgow from 19th–22nd August 1985. The workshop will consist of three days of lectures, discussions, demonstrations and practical experimentation in various aspects of sequencing.

Further details, regarding course content, workshop fee and accommodation will be available on request.

Application and inquiries to M McAlevy, GIBCO BRL, Trident House, Renfrew Road, Paisley, Scotland. Tel. No. 041-889-6100 (7045)V

STUDENTSHIPS

**UNIVERSITY OF
BIRMINGHAM AND THE
GENERAL HOSPITAL
SERC-CASE STUDENTSHIP**

Applications are invited for a studentship in collaboration with Beechams Research Laboratories to investigate the pathogenesis and treatment of chronic bronchial sepsis. The work will involve developing immunoassays for antibiotics, — Lactamases and host immune response to lung pathogens.

Send a CV with names and Tel Nos of two referees as soon as possible to Dr D Burnett, Clinical Teaching Block, The General Hospital, Steelhouse Lane, Birmingham B4 6NH. Tel 021-236-9922.

(7023)F

**UNIVERSITY OF
NOTTINGHAM**

**DEPARTMENT OF PHYSIOLOGY
AND ENVIRONMENTAL SCIENCE
SERC-CASE
POSTGRADUATE
STUDENTSHIP**

A studentship is offered in collaboration with Asmer Seeds Ltd. The research will involve the production of novel interspecific and intergeneric hybrids between the tomato and wild relatives. Particular attention will be given to developing tissue culture techniques for rescuing hybrid embryos between the tomato and the potato relative, *Solanum tuberosum*. The aim is to provide new sources of cold tolerance and frost resistance for the tomato breeder. The studentship is tenable for 3 years from 1st October, 1985; with a view to reading for the degree of PhD. The student will be expected to have obtained an upper second class honours degree.

Applications, including a curriculum vitae should be sent without delay to Dr I B Taylor, Dept of Physiology and Environmental Science, University of Nottingham School of Agriculture, Sutton Bonington, Nr Loughborough, Leics. (7034)F

**UNIVERSITY COLLEGE
CARDIFF**

**DEPARTMENT OF PHYSIOLOGY
PhD STUDENTSHIP**

Applications are invited for a Research Studentship in a project concerned either with: a) Synaptic interactions in the central visual system. b) Modulation of sensory transmission through thalamic relay nuclei.

The projects will take place in well equipped MRC funded laboratories and will offer access to a wide range of the latest techniques. Applicants should have a First or Upper Second Honours Degree in a relevant scientific subject. Those interested should apply to Professor A M Sillito, Department of Physiology, University College, PO Box 78, Cardiff CF1 1XL, enclosing a brief Curriculum Vitae and the names and addresses of two referees. Informal enquiries can be made to Professor Sillito by telephone on 0222 44211 Ext 2062, and potential applicants will be welcome to visit the laboratories.

(7025)F

**THE CHINESE
UNIVERSITY OF
HONG KONG
Faculty of Medicine
Department of
Biochemistry**

Applications are invited for a Royal Hong Kong Jockey Club Research Studentship, commencing September 1985, leading to the degree of Ph.D. The successful candidate will join an active research group investigating the molecular basis of amino acid transport in mammalian erythrocytes and other cell types.

Applicants should have a first or upper second class honours degree in Biochemistry or a related discipline and should have a strong interest in membrane biochemistry. Candidates with an existing postgraduate qualification (M.Sc. or M.Phil.) are encouraged to apply.

Applications, containing a CV and names and addresses of two professional referees, should be sent as soon as possible to Dr D.A. Fincham c/o Physiological Laboratory, Downing Street, Cambridge CB2 3EG, U.K., from whom further particulars may be obtained (Tel: Cambridge (0223) 64131, Ext. 223). (W2011)F

Continued on page 22

FELLOWSHIPS

**EMBO
European Molecular Biology Organization
LONG TERM FELLOWSHIPS IN
MOLECULAR BIOLOGY
Autumn 1985 — Awards**

Next deadline: 15 August 1985

EMBO long term post-doctoral fellowships are awarded to promote the development of molecular biology and allied research in Europe and Israel. To be eligible a candidate must hold a doctorate degree and the exchange must involve a laboratory in Western Europe or Israel. EMBO fellowships are not, however, awarded for exchanges between laboratories within any one country. Long term fellowships are awarded initially for one year, but depending on a review of progress, they may be renewed for a second year. In cases of exceptional scientific merit renewal for a third year is possible. The fellowship comprises a return travel allowance for the fellow and any dependents and a stipend and dependents' allowance.

Since the selection procedure may include an interview, candidates are requested to respect the deadline for complete applications which is 15 August 1985. Successful candidates will be notified of their awards immediately after the meeting of the selection committee, which is on 21 October 1985.

Application forms and further details may be obtained from Dr. J. Tooze, Executive Secretary, European Molecular Biology Organization, Postfach 1022.40, 69 Heidelberg 1, F. R. Germany. (W1893)E

**THE ANIMAL VIRUS RESEARCH INSTITUTE
POST-DOCTORAL FELLOWSHIP IN
MOLECULAR BIOLOGY
(Funded by The Overseas Development
Administration)**

Applications are invited for a 4 year fellowship (beginning October 1st 1985) to work on analysis of the genome of capripox viruses and their development for use as a eukaryotic vector system.

The successful candidate will work with Dr Donald Black in The Division of Molecular Biology. He/she should have a degree in Biochemistry or a related subject and a PhD. Experience with expression systems and eukaryotic gene analysis would be an advantage.

The salary will be in the scale £7,788 to £10,541 (Higher Scientific Officer Grade). Sick leave and non-contributory superannuation schemes, 22 days annual leave. Accommodation may be available. The AVRÍ is an equal opportunity employer.



Application to include a curriculum vitae and the names of two referees should be sent as soon as possible to the Secretary, Animal Virus Research Institute, Ash Road, Pirbright, Woking, Surrey GU24 0NF. Tel. 0483 232441. (7010)E

**ROYAL FREE HOSPITAL
SCHOOL OF MEDICINE
(University of London)
ACADEMIC DEPARTMENT OF
PHARMACOLOGY
JUNIOR RESEARCH
FELLOWSHIP**

Applications are invited from graduates with good honours degrees in Pharmacology or related subjects for a Junior Research Fellowship commencing in October 1985. The successful candidate will be expected to register for a higher degree in the University of London and work in endocrine pharmacology. The conditions of the fellowship are similar to those of SERC/MRC studentships.

A curriculum vitae and names of two referees should be sent to Professor J R Hodges, Academic Department of Pharmacology, Royal Free Hospital School of Medicine, Rowland Hill Street, London, NW3 2PF. (7005)E

**POSTDOCTORAL
FELLOWSHIP IN MOLECULAR
BIOLOGY/ BIOCHEMISTRY**

Position available in Department of Microbiology at SUNY Stony Brook to study Adeno-associated virus genetics, vector construction, immunology or DNA replications. Send CV and references to Dr Nicholas Muzyczka, University of Florida, College of Medicine, Box J226 JHMC, Gainesville, FL 32610. (NW1901)E

Please mention

nature

when replying to
these advertisements



ZWO space research organisation of the Netherlands
Laboratory for space research Groningen

post doctoral fellowship

in sub-mm heterodyne techniques for
astronomy.

The Laboratory has a 2-4 year position for a post doctoral astronomer/instrumentalist to participate in the development, construction and application of sub-mm heterodyne equipment for use at ground based and balloon borne telescopes. Efforts are concentrated on the development of superconducting mixers (sis and jcn) at frequencies between 300 and 1000 ghz, and on mixer arrays.

Experience in the field of sub-mm astronomy and related experimental techniques is required, preferably with mixer development.

The candidate will be employed by the Netherlands organization for the advancement of pure research (Z.W.O.). The salary will be according to the Dutch government scales for research positions.

Applications should be sent to the head of the personnel office, bureau SRON, Beneluxlaan 21, 3527 HS Utrecht, The Netherlands and must include a curriculum vitae, a List of publications, research plans for the period at the space research Laboratory and should arrive by August 1, 1985. Candidates have to provide names of two referees.

Detailed information can be obtained from Dr. Th. de Graauw, Laboratory for space research, Groningen, P.O. Box 800, 9700 AV Groningen, The Netherlands. Phone 050-116695.

(W1985)E



National Research Fellowships QUEEN ELIZABETH II AWARDS

The Australian Government has established the National Research Fellowships — Queen Elizabeth II Awards to replace the previous Queen Elizabeth II Fellowships and Queen's Fellowships Schemes. Under this Scheme, 15 awards will be made annually. Fellowships may be taken up in industry, universities, colleges of advanced education or government research organisations in Australia and are normally for two years. Tenure of a Fellowship is expected to commence within nine months of the announcement of the award. The awards are for high quality research work in one of the three categories described below:

1. **FUNDAMENTAL RESEARCH**
2. **INDUSTRY BASED RESEARCH**
3. **RESEARCH IN PRIORITY AREAS OF NATIONAL INTEREST**

Priority areas of National Interest may include:

- Research and development into products and processes, marketing, trade and social and legal issues concerning high technology areas such as:
 - energy technology
 - information technology and communications
 - materials technology
 - biomedical technology
 - biotechnology
 - raw materials processing
- Manufacturing technology
- Productivity
- Income, wealth and social inequality
- Social and economic issues affecting training and employment with particular regard to those affecting women and youth
- Occupational safety and health
- Social and economic implications of ageing
- Aboriginal Affairs

- Provision of services e.g. welfare, transport, health care
- Marine Sciences and Technologies

QUALIFICATIONS

Fellowships will be awarded to persons holding a Ph.D or equivalent qualifications, or equivalent research or professional experience in one of the disciplines available under the National Research Fellowships Scheme. The decisions regarding equivalent qualifications will be made on a case by case basis. Recommendations for the award of Fellowships will be made to the Minister for Science by an Advisory Committee. Awards will, in general, be restricted to applicants who are not more than 30 years of age when applications close.

ELIGIBILITY

Fellowships will be awarded on merit to suitably qualified persons.

SALARY

\$27,414 (Australian) per annum — increased to \$29,763 per annum at age 28 years.

TRAVEL EXPENSES

Necessary travel expenses are payable.

APPLICATIONS Persons interested in applying for the above fellowships should obtain application forms and a statement of the conditions of award from: Executive Officer, National Research Fellowships Scheme, Department of Science, P.O. Box 65, Belconnen, A.C.T. 2616, Australia.

The next round of awards will be announced in December 1985. Applications received after the closing date of 10 September, 1985 will not be considered. People who applied for the earlier round which closed on 17 May 1985 will also be considered for this round and need not reapply. (W2003)E

UCG Coláiste na hOllscoile Gaillimh

UNIVERSITY COLLEGE GALWAY
IRELAND

POST-DOCTORAL RESEARCH FELLOWSHIPS (5)

Applications are invited for post-doctoral research fellows to work on the following topics:

- (1) "The Cloning and Analysis of the Estrogen Receptor Gene". This project is in collaboration with groups in Strasbourg and Chicago and is funded by the Cancer Research Advancement Board of Ireland. Interested applicants should contact Dr Frank Gannon, Department of Microbiology.
- (2) "The Direct Analysis of the Fertilizing Ability and Chromosomal Constitution of Bovine Spermatozoa" funded by the National Board for Science and Technology. Applicants with interests in reproductive biology, physiology, cytogenetics or a related field should contact Professor James A. Houghton, Department of Microbiology.
- (3) "Molecular Genetics Studies on Amino Acid Producing Strains of *Corynebacteria*" funded by the National Board for Science and Technology. Interested applicants should contact Professor L.K. Dunican, Department of Microbiology.
- (4) "Molecular Cloning of $\Delta 12$ desaturase" funded by the National Enterprise Agency of Ireland. Applicants should contact Dr D.R. Headon, Department of Biochemistry.
- (5) "Characterization and Molecular Cloning of Bovine INHIBIN" funded by the National Board for Science and Technology. Dr D.R. Headon, Department of Biochemistry.

Applications including Curriculum Vitae should be addressed directly to the project leader at University College, Galway, Ireland, Tel. No. (091) 24411, from whom further details of the project may be obtained.

(7035)C

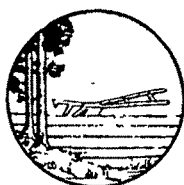
UNIVERSITY OF READING

DEPARTMENT OF MICROBIOLOGY POST-DOCTORAL RESEARCH FELLOW

Applications are invited for this Fellowship which is supported by MRC and is for a fixed period of two years from 1 September 1985 with possible extension. The project under Professor J W Almond, is to study the molecular basis of poliovirus neurovirulence using infectious, cloned cDNAs. The investigation forms part of a larger programme concerned with the de-

velopment of new prophylactic and therapeutic agents against medically important picornaviruses. Previous experience of recombinant DNA techniques and/or of virology would be an advantage. Starting salary will be up to £9,390 pa.

Candidates (PhD) should apply for application forms, quoting Ref R 30A, to Personnel Officer, University of Reading, Whiteknights, PO Box 217, Reading, RG6 2AH, telephone (0734) 875123 ext 233. Dr Almond, currently at the University of Leicester, is Professor elect of the Department of Microbiology at the University of Reading. (7021)E



THE MACAULAY INSTITUTE FOR SOIL RESEARCH SWAP RESEARCH STUDENTSHIP

Applications are invited from candidates who hold, or expect to obtain in 1985, a good honours degree in physical or analytical chemistry for a research studentship at the Macaulay Institute.

Methods for the determination, in surface and soil waters, of the forms of aluminium toxic to fresh water biota will be evaluated and new methods for the speciation of aluminium, including the use of HPLC fractionation with graphite furnace atomic absorption spectrometry, developed. These methods will be applied to water samples from catchment studies of the interaction of acid rain with vegetation and soil in modifying the acidification of streams and the release of aluminium into them. This 3-year study will be part of the Surface Water Acidification Programme (SWAP) under the auspices of The Royal Society, The Norwegian Academy of Science and Letters and The Royal Swedish Academy of Sciences. The holder of the studentship will be registered for the degree of Doctor of Philosophy of the University of Aberdeen.

Further information may be obtained from The Secretary, The Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen, AB9 2QJ, to whom applications giving the names and addresses of three referees should be sent by 22 July 1985. Quote Ref. SWAP/5/85. (6990)F

UNIVERSITY OF DUNDEE

DEPARTMENT OF BIOLOGICAL SCIENCES POSTGRADUATE STUDENTSHIPS IN BOTANY

Applications are invited for two SERC-funded studentships tenable from October 1985:

(a) an 'ear-marked' award to Dr Jonathan Weyers to support research funded by SERC grant GR/D/03680 on solute relations of stomatal guard cells. The project will involve the preparation of epidermal strips and guard cell protoplasts, and the identification and quantification of solutes involved in stomatal movements using a range of techniques (including emission flame spectrometry, radioassay by scintillation counting, TL, HPL, and GL chromatography, enzymic analyses and electron microscopy).

(b) an award for research into oxygen build-up in cells of aquatic phototrophs to be supervised by Professor J A Raven. Quantification of this build up in plants with a 'CO 22 3 concentrating mechanism' would involve use of a variety of O 22 3 microelectrode, mass spectrometric and gas chromatographic techniques. Subsequently, the significance of the build-up for photosynthesis, and other, metabolism could be explored.

Applicants for the above studentships should have or expect a good Honours degree in botany or a related subject. Applicants, stating experience, should be sent to either Dr J D B Weyers or Professor J A Raven, Department of Biological Sciences, University of Dundee, DUNDEE DD1 4HN, Tayside, UK. (6991)F

VACANCIES

PhD STUDENTSHIPS

Northern Carbon Research Laboratories,
School of Chemistry,
Bedson Building,
University of Newcastle upon Tyne,
Newcastle upon Tyne NE1 7RU

Two CASE studentships are available in the above Laboratories, leading a PhD by research.

1 DEVELOPMENT OF CARBON-CARBON COMPOSITES

Supported by SERC and T & N Materials Research Ltd, Lancashire.

The aim of the research is to develop a new generation of carbon-carbon composites based on an understanding of liquid crystals formed from pitch.

2 COMBUSTION OF LIGNITES IN POWER GENERATION

Supported by SERC and Babcock Power Ltd, London. The aim of the research is to characterise lignite coals by FTIR, adsorption methods, reflectance microscopy and combustion behaviour in a transient flow reactor.

The studentships are suitable for graduates (BSc Hons) in Chemistry, Geology, Materials Science.

Applications and requests for the further details should be sent to Dr H Marsh or Dr I A S Edwards at the above address (telephone 0632 328511 Ext 3085/3084). No special forms are needed. Enclose a CV. (7006)F

UNIVERSITY OF READING

AGRICULTURAL BOTANY DEPARTMENT POSTGRADUATE RESEARCH STUDENTSHIPS

Applications are invited for two studentships to commence in October 1985

(a) A MAFF STUDENTSHIP

to work on the agro-ecology of *Phalaris paradoxa*, an annual grass weed which has recently become a problem in arable production in some parts of Eastern England. Studies of germination requirements, control and genetic variation will be undertaken. The work will be supervised by Dr D S H Drennan.

(b) An ODA STUDENTSHIP

to work on physiological and biochemical aspects of ripening in commercially important tropical fruit. The work will be jointly supervised by Dr A K Thompson (Tropical Development and Research Institute, London) and Dr P John.

Applicants should have at least an upper second class degree in an appropriate subject. Telephone enquiries can be made to Dr Drennan (0734 875123 ext 7923) or Dr John (Ext 7900). Applications, including a curriculum vitae and names of 2 academic referees, should reach Dr Drennan or Dr John before 19 July 1985. (7018)F

UNIVERSITY OF ST ANDREWS

DEPARTMENT OF BIOCHEMISTRY AND MICROBIOLOGY AN SERC STUDENTSHIP

is available in the laboratory of Dr W John Ingledew, Department of Biochemistry and Microbiology.

The project is on the 'Relationship between the function and organisation of the respiratory chains of *Escherichia coli*', and is linked with other continuing projects in the laboratory. The techniques involved in the project will concentrate on the use of stopped and quenched flow kinetics methods, combined with detection by optical spectroscopy and electron paramagnetic spectroscopy. Work on the determination of anisotropic arrangement of the components in the cell membrane will also be pursued. For reference see Ingledew and Poole (1984) Microbiol Rev (48) 222 — 271.

Applicants should hold or expect to hold at least an upper second class honours degree in one of the following subjects or a related subject — Biochemistry, Biophysics, Microbiology, Chemistry.

Application and requests for further details to Dr W J Ingledew, Department of Biochemistry and Microbiology, University of St Andrews, St Andrews, Fife KY16 9AL. (7036)F

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Tel. (0483) 571281 Ext. 577.
Telex 859331.

(7039)L

ASSISTANTSHIPS

UNIVERSITY OF GLASGOW
DEPARTMENT OF BIOCHEMISTRY
Applications are invited for a SERC funded

POSTGRADUATE RESEARCH ASSISTANTSHIP

to investigate aspects of the Biosynthesis, import and assembly of pyruvate dehydrogenase multi-enzyme complex in mammalian cells. The work will be part of a research programme supervised by Dr J Lindsay.

Suitable applicants will have an opportunity to study for a PhD; some experience in tissue culture, protein purification and immunological techniques would be an asset. The salary will be on the 1B scale plus USS. The appointment, which will begin on 1st October, 1985, or soon as possible thereafter, is for a maximum of 3 years.

Applications (one copy), giving the names of two referees, should be sent to Dr J G Lindsay, Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ, from whom further details may be obtained. (7057)J

UNIVERSITY OF DURHAM

DEPARTMENT OF CHEMISTRY

Applications are invited for postdoctoral

SENIOR RESEARCH ASSISTANTSHIP

tenable for two years, from October 1985, to work with Dr J Munro and Dr D Parker on the synthesis and characterisation of electroactive, polymeric thin film. Applicants should have a strong background in electrochemistry; some knowledge of surface chemistry is also desirable. The starting salary is £7,520 — £8,920 on RA 1A plus superannuation (under view).

Applications (3 copies) naming three referees should be sent by July 1985 to The Registrar, Science Laboratories, South Road, Durham DH1 3LE, from whom further particulars may be obtained. (7032)

UNIVERSITY OF NOTTINGHAM
Nottingham NG7 2RD
POSTDOCTORAL RESEARCH ASSISTANTSHIP (Grade 1A)

DEPARTMENT OF ZOOLOGY
Applications are invited for a post-doctoral research assistantship (Grade 1A) to work with Dr D I de Merai on a crystallin gene expression in transdifferentiating chick embryo cell cultures. The techniques to be used will include cloning, Northern blotting, primer extension analysis and nuclear runoff transcription assays. Molecular biologists with experience in some or all of these techniques would be suitable for this post, which is funded by ARC. The appointment will be made at the bottom of the 1A scale, and the duration will be three years, starting 1st January 1986.

Applications, giving a full CV and names and addresses of two referees, should be sent to Dr de Merai at the above address by 15th September 1985. (7031)P

UNIVERSITY OF OXFORD
Dyson Perrins Laboratory
STREPTOMYCES BIOSYNTHETIC ENZYMES: Cloning and Application to Novel Antibiotic Synthesis

Applications are invited for a post-doctoral research assistantship to work on the cloning, isolation and characterisation of antibiotic biosynthetic enzymes from Streptomyces and their application to the enzymatic synthesis (in vitro and in vivo) of novel antibiotics. This work will be performed in collaboration with Dr K F Chater, complementing current research on streptomyces genetics at the John Innes Institute in Norwich. Candidates with experience of molecular biology, enzymology or bioorganic chemistry are preferred. The appointments are on the RA1A scale with starting salary up to £8,920 pa plus USS according to age and experience and would be initially for one year from October 1985 with the possibility of extension.

Applications with curriculum vitae and the names and addresses of two referees should be sent as soon as possible to Dr A J Pratt, Dyson Perrins Laboratory, South Parks Road, Oxford, OX1 3QY. (7043)P

APPOINTMENTS WANTED
advertise your qualities and qualifications through the most influential science weekly in the world at a special reduced cost of \$8 per line. Personal Box Numbers \$10.00). Advertisements must be pre-paid and sent to: *Nature Classified (Appts Vtd)*, 65 Bleecker Street, New York, NY 10012. (890)B

THE OPEN UNIVERSITY
Faculty of Science
RESEARCH ASSISTANTSHIP ON PLATINUM GROUP ELEMENTS

Applications are invited for an EEC-funded research assistantship to study the process controlling the distribution of the Platinum Group Elements in ophiolite complexes. Candidates will be expected to hold a good first honours degree in geology and have specialised to some extent in geochemistry. The assistantship is available immediately for 1 year and may be extended for a further 2 years. Registration for an O U higher degree may also be possible.

Salary will be on the 1B Scale for Research and Analogous Staff (£6,600 — £8,920 pa under review). Starting salary will depend on age and experience but will not exceed £7,980 pa.

Application forms and further particulars are available from Dr J W R Smith (4850/1), Administrative Assistant Science (4850/2), Faculty of Science, The Open University, Walton Hall, Milton Keynes, MK7 6AA or telephone Milton Keynes (0908) 653993: there is a 24 hour answering service on Milton Keynes 653868.

Closing date for applications: 19th July. (7014)P

UNIVERSITY OF DUNDEE
DEPARTMENT OF BIOCHEMISTRY
POST-GRADUATE RESEARCH ASSISTANTSHIP

Applications are invited from graduates with a good honours degree or undergraduates who expect to obtain at least an upper second class honours degree this year for a British Diabetic Association supported post.

The successful applicant will work with Dr A Burchell on a project investigating the genetic regulation of hepatic microsomal glucose-6-phosphatase during diabetes. The project will involve extensive use of protein chemistry and recombinant DNA technology, and applicants should have a strong interest in one of these disciplines.

The post is for three years duration starting late Summer 1985 and the salary will be on the Research & Analogous Range 1B scale (£6,600 — £7,520 per annum).

Informal enquiries may be made to Dr A Burchell (Tel 0382 23181 ext 4237).

Applications containing a full Curriculum Vitae and the names and addresses of three referees should be sent to the Personnel Officer, The University, Dundee, DD1 4HN, as soon as possible. Please quote Ref EST/76/85J. (6998)P

CONFERENCES & COURSES

ADVANCED COURSE IN FLOW CYTOMETRY; ANALYSIS AND FLOURESCENCE ACTIVATED CELL SORTING
Paris, September 9-14, 1985

Sponsored by: The Cancer Research Association (ARC) — Jacques Crozemarie, President, and the "Centre Technique pour le soutien de la Recherche sur le Cancer des Laboratoires et Formations du Centre National pour la Recherche Scientifique" (C.N.R.S.)

A Course in Flow Cytometry will be offered with special emphasis on cell biology. Themes will include Multiparameter cell sorting, cell lineage studies, tumor cells characterization, gene amplification selection, cloning of genes by transfection, somatic cell genetics, hybridoma variant selection, liposome endocytosis, cell cycle analysis. Brd U and cytokinetic studies, mathematical modeling of the cell cycle, computer system for multiparameter data analysis, cytochemistry and new fluorochromes, karyotype analysis, pumped dye lasers, emission anisotropy.

The Course will consist of lectures, laboratory demonstrations and problem solving sessions.

Course Directors: Harry Crissman (Los Alamos National Laboratories), Claude Rosenfeld (INSERM, Villejuif, France)

Organizers: L.A. Herzenberg (Stanford University, USA), P. Le Bouteiller (Centre d'immunologie de Marseille-Luminy, France), Z. Mishal (C.N.R.S., Villejuif, France), M. Monsigny (Centre de biophysique moléculaire, Orleans, France), J.L. Roti-Roti (Washington University of St. Louis, USA).

Lecturers will include the above plus: B. Bagwell, S. Cram, Z. Darzinkiewicz, P. Dean, M. Goldberg, J. Gray, P. Horan, C. Julien, M. Loken, P. Manoni, H.S. Micklem, K. Muirhead, D.R. Parks, A. Truneh.

Course tuition: \$500 (5 000 FF) includes laboratory fees, manual, coffee breaks, lunches and an optional Flow Cytometry Certificate.

Send C.V. and tuition deposit \$50 (500 FF) to the local Course Director (max. 50 persons).

Dr. Claude Rosenfeld,
INSERM Unite 253,
Groupe Hospitalier Paul Brousse,
16 bis avenue Paul Vaillant Couturier,
94804 Villejuif, France.

Application deadline June 30 1985, priority given to early applicants. (W1919)C

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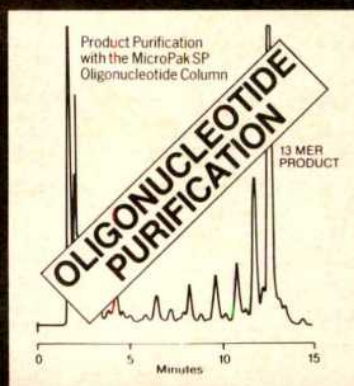
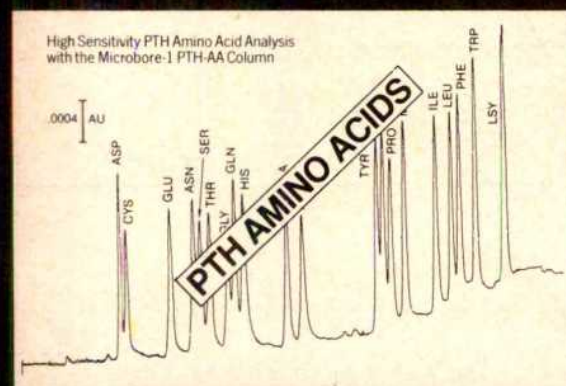
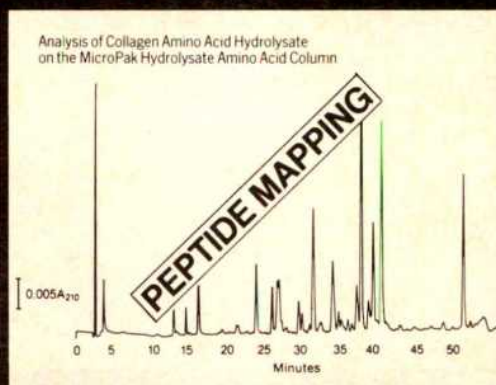
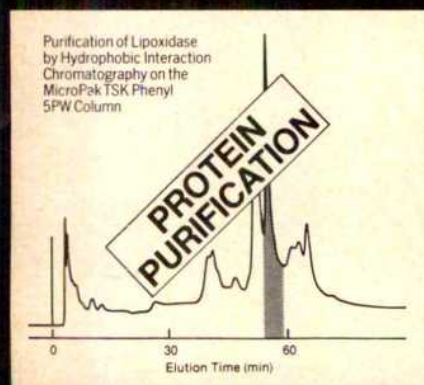
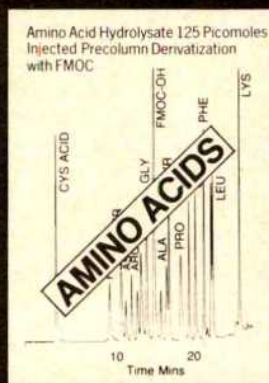
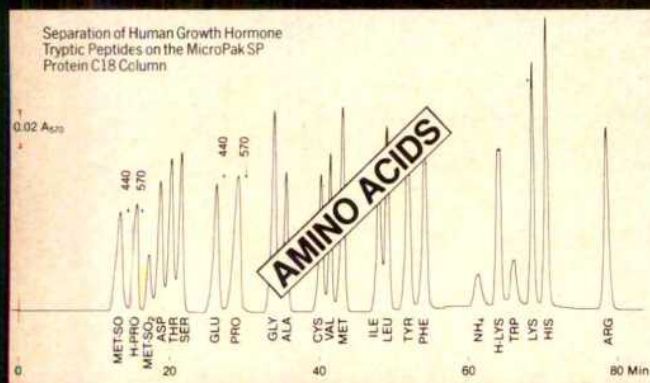
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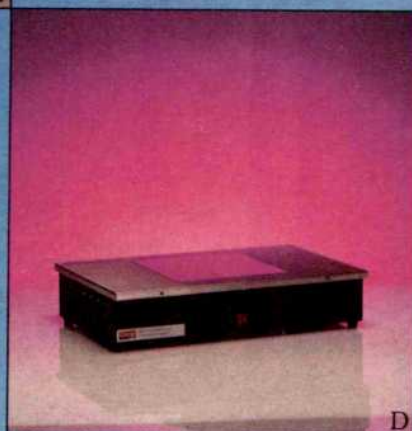
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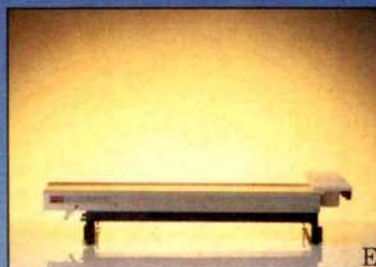
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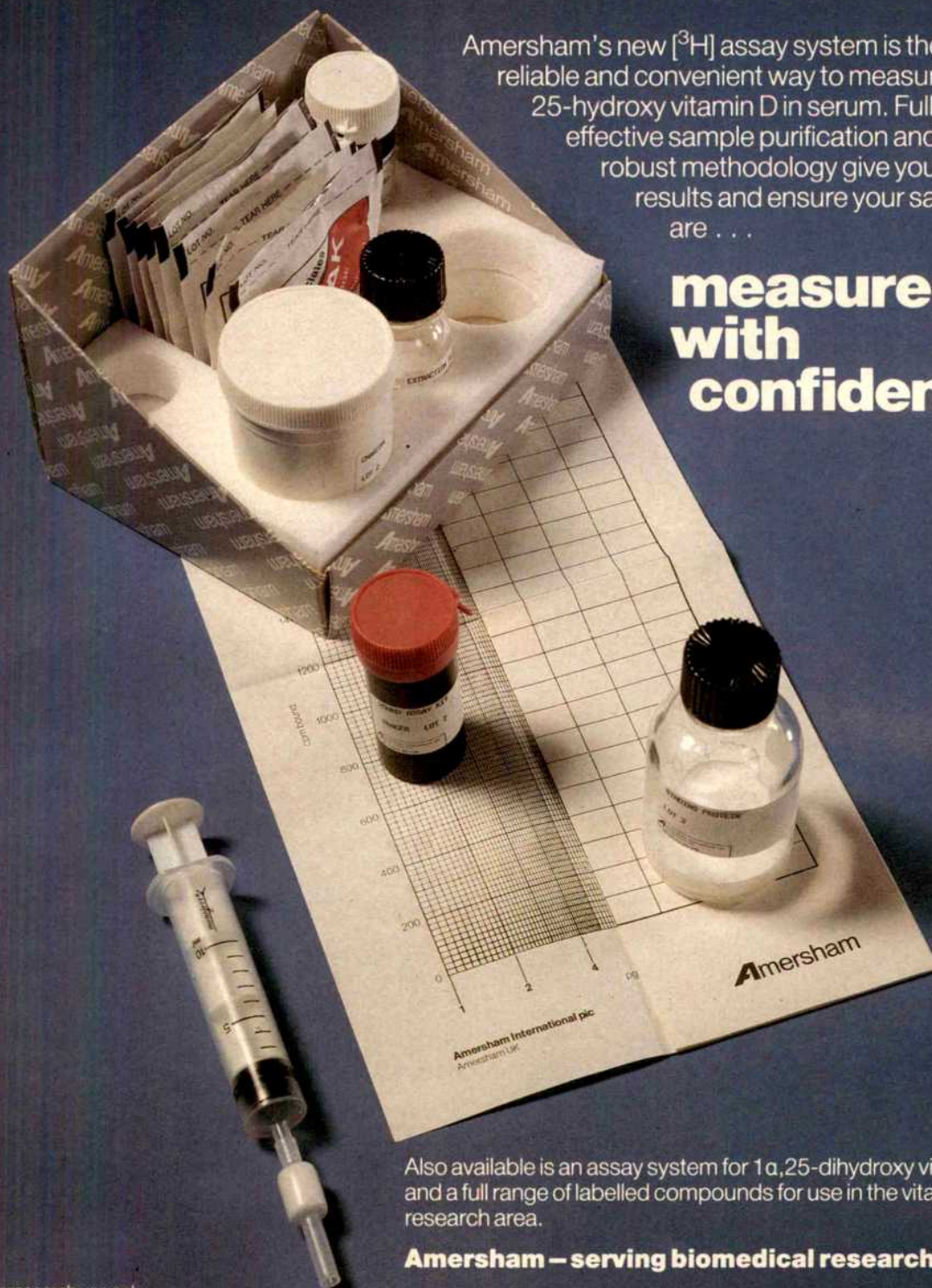
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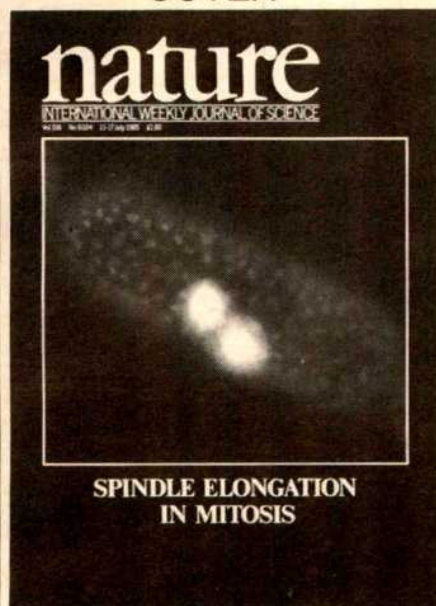
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The mitotic spindle is responsible for the equipartition of chromosomes during cell division. The cover shows a chain of dividing diatom cells stained with DAPI to visualize the separating masses of chromosomes. The letter beginning on page 170 shows that isolated diatom spindles are able to undergo spindle elongation *in vitro*.

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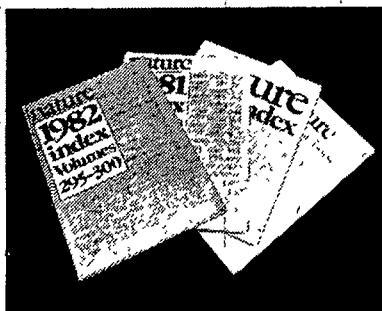
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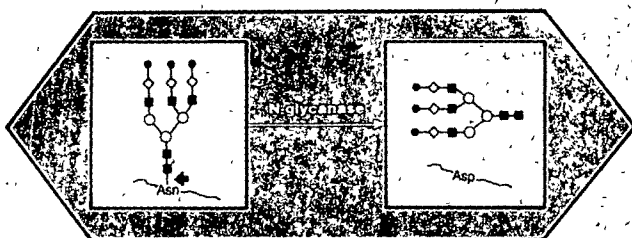
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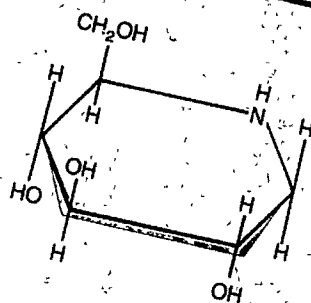


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ENDOGLYCOSIDASE

1-DEOXYNOJIRIMYCIN



Activity:
Reference:

Inhibitor of glycoprotein processing
JBC, 257 : 14155-14161 (1982).

O-GLYCANASE

(Endo- α -N-acetylgalactosaminidase)



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Electronics in trouble worldwide

Two years of upheaval in the market for microprocessor computers have now been followed, in Britain, by stockholders' uneasiness about electronics giants. Stoicism is called for.

WHAT has gone wrong with the electronics industry? For the past decade, as the benefits of integrated semiconductor devices have become apparent not merely in the design of novel equipment such as personal computers but also in telecommunications equipment serving a traditional purpose in a novel way, prophets have been singing the praises of what the future will be like, and those with money to invest have believed what they have heard. The recent past has been traumatic for them. In the United States, the troubles began more than two years ago, when mushroom companies, large as well as small, found that their financial accounts were having to be written mostly in red. Many have since gone out of business, others have been bought up cheaply by their competitors. It is not surprising that the New York stock markets now take a more cautious view of the attractions of investing in electronics companies. Nor is it surprising that the rot has now spread outside the United States, to Britain in particular. Some manufacturers of personal computers (such as the Wales-based Dragon) went to the wall almost as soon as they began. More recently apparently solid companies have run into trouble. Acorn, the benefits of an arrangement with the BBC notwithstanding, has had to be rescued by Olivetti but may yet fail, Sinclair has been bought up by an offshoot of Mr Robert Maxwell's Pergamon Press. Now, to the general consternation, some of the giants of British electronics manufacturing are deeply offending their owners. Stockholders are asking whether the future has been indefinitely postponed.

Rail fever

Small comfort though it may be, the present trouble in electronics is not without precedent. British economic history includes one of the most vivid illustrations of how the rapid development of a new technology may be a mixed blessing for those tempted to stake their fortunes on it—the development of the railway system. During the period from 1840 to 1870, George Stephenson's example served as a powerful stimulus of investment in new railway companies. In the event, many were geographically too confined to be viable except as adjuncts of larger companies, others were discovered to have grossly overestimated the markets for which they were established and went out of business, still others (as the railway map of Britain in the 1950s shows clearly) duplicated what other companies were about. By the end of the nineteenth century, the Victorian railway boom had enabled some of those who backed it to make great fortunes, but had robbed many others of their shirts. The recent upheaval among manufacturers of personal computers has clearly run along very similar lines, but the time-scale of events has been dramatically foreshortened.

In retrospect, the course of events should nevertheless be no surprise. Companies such as Atari grew quickly on the back of a fashion of the late 1970s, young people's interest in playing

games of a novel kind on television screens. But soon after Atari had been bought by Warner Communications, the gingerbread began to go stale as the fashion changed. Warner itself seemed for a time financially vulnerable. Among manufacturers of more seriously intended personal computers, there have been two recurrent technical problems, a persistent tendency to underestimate the length of time needed to turn a good idea into a workable (and saleable) device (software as well as hardware) and the maddening issue of the degree to which one machine is compatible with another.

Service

The persistent misjudgement of market needs, especially by the smaller manufacturers, is however much more relevant. In a field so novel, for example, the need for after-sales service to help customers use what they had bought should have been plain. Yet many companies, some of them casualties of the upheaval of the past two years, appear to have left that need out of account. But is not providing such a service, especially internationally, bound to be both intricate and expensive? That is what the smaller manufacturers have been saying. Of course. But what purpose is served in the long run by marketing even technically excellent products without providing what must be regarded as services essential to their proper use? In the unsophisticated markets of a few years ago, many smaller companies calculated that buoyant sales of low-priced hardware would give them a niche from which they might afterwards expand. One of the outstanding characteristics of this part of the computer market is that the cost of hardware may be only a fraction of the total, at least when some account is taken of the time unsophisticated users must spend on learning to use what they have bought. This truth, an awareness of which is now spreading, no doubt accounts for the recent slump in sales of even technically excellent machines. Investors will no doubt take note.

The more surprising recent faltering of confidence in major British companies must be differently explained. Part of the trouble is worldwide. In New York, even IBM's share price has not continued its buoyant upward surge as people have begun to realize what should have been evident, that the years immediately ahead are bound to see both high investment in research and development and fierce competition in the marketplace. The acquisition of the communications company MCI by IBM two weeks ago will, for example, bring about novel competition with established providers of telecommunications services, in which the profits of all concerned may suffer, to the general benefit of the users of these services. That, at least, is what should happen in the United States and possibly elsewhere if the regulators and legislators who have recently been deregulating the communications market do not lose heart.

The British scene is, as always, muddier, and should be an object-lesson both for governments and investors. One powerful influence has been the conversion of British Telecom from a public communications monopoly to a private near-monopoly. The immediate result has been to destroy the market for all except the most up-to-date capital equipment in the British network. Suppliers of analogue exchange equipment, on the development of which British Telecom's predecessors and the major British suppliers were engaged as recently as 15 years ago,

Biological manuscripts

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are now having to close manufacturing plant (which they might have expected), while the purchaser under whose umbrella they used to shelter now plans to play the international market for digital exchange equipment. The moral is that private companies that become over-dependent on a single customer (in Britain, Plessey and STC seem to be worst hit) are vulnerable in two respects: technically their pattern of activity is constrained, financially they depend on the whim of others.

There are also more immediate problems. The general depression and competitiveness of the computer market has made STC's purchase of the British company ICL seem less of a bargain than it did last year. Fierce international competition for general-purpose semiconductor chips, which has meant that prices have fallen by a factor of five within a year, has similarly afflicted the British company Thorn-EMI, the purchaser last year from the British government of Inmos, the semiconductor company. Those with an interest in these questions are now wondering which chairmen will be forced to leave which companies. Those with longer memories may recall the curious and still unexplained outburst last month by Lord Lucas, a government spokesman in the House of Lords, who surprised his audience with a recipe for the amalgamation of British electronics companies. The most obvious candidates for shotgun marriages are the companies whose balance-sheets (and share prices) have been hit by increased competitiveness of the general electronics market, but even companies such as GEC (no relation of US General Electric), which have stuck to customized semiconductor electronics for the most part, have disappointed their shareholders while maintaining their profits.

The outcome of this period of upheaval cannot, of necessity, be predicted. Indeed, even companies that have been hard hit in the past few weeks are strong industrial organizations, well able to ride out the storm. But the best-provided must be those whose interests span more than electronics pure and simple. They are best placed to swallow one or more of the others. The snag, for disappointed investors, is that in the present climate they may not wish to do so.

If the immediate future cannot be foretold, the more distant future is fortunately more predictable. Electronic computers with hugely different capacities are now well-established and productively used, while there is likely to be a lull of several years before machines of radically different architecture are working tools. So too are the technologies of telecommunications. But the marriage of the two fields has so far been half-hearted. That is why, with the increased competition between the giants of the two industries, the next decade should throw up novel opportunities for the smaller fry to become suppliers of specialized equipment; there should be plenty of niches for other companies to fill. Investors should know, however, that capital investment in research and development will be more than ever needed if they are to be filled. Meanwhile, ambitions to overtake the giants by sheer cleverness (which is not in short supply) should be surpassed. □

OPEC in the doldrums

OPEC is going through a bad patch, but remains a potentially powerful cartel.

OPEC, the Organization of Petroleum Exporting Countries, seems these days to stagger from one crisis to another. Last weekend's meeting in Vienna was no exception. After three days of talk, OPEC failed to persuade itself that there should be a system of production quotas that would restrict the amounts of oil that could be sold on world markets. Even the proposal that the total volume of OPEC production should be reduced by 7 per cent, unenforceable without production quotas, is only a formality, setting a ceiling (of 14.9 million barrels a day) believed to be higher than OPEC members now sell collectively. It is always possible that the economic climate may change before the next formal meeting of the organization, scheduled to begin on 22 July, but the prospects (for OPEC members at least) are

not bright.

Why is OPEC now apparently so weak when it seemed, in 1973 and 1979, that it could fix whatever price it chose for a barrel of crude oil and still keep its members' oil revenues at levels that satisfied their needs for overseas currency? The time-lag between 1979 and now is significant and the explanation is almost banal. The sudden jumps of price in the 1970s were shocks to the economies of the oil consumers. The short-term effects included all kinds of economic problems, stagflation in particular. One of the long-term consequences has been simple old-fashioned price resistance. Customers reconciled to prices of between \$10 and \$12 a barrel (the official price of Saudi Arabian crude oil between the first and second shocks) have changed their ways, and have taken steps to use less oil. In retrospect, it is unlikely that OPEC would have dared put the price above \$30 a barrel in 1979 if it were not that Iranian production had been halted. Since then, however, the trend of prices for the oil products sold on the Rotterdam market has been steadily downwards, in dollars and without allowing for inflation.

The driving force behind these dramatic changes is the way in which the oil consumers have managed to economize in its use. It is almost as if OPEC's 1979 round of price increases had been designed to bear out the contention of the late Shah of Iran that higher prices were the best way of ensuring that oil would be left in the ground for "future generations" to use as a source of chemicals.

The industrial trends are neatly illustrated in British Petroleum's latest *Statistical Review of World Energy*, published last month. Between 1970 and 1984, the consumption of primary energy in the United States for each unit of gross domestic product (GDP) fell by some 17 per cent. In the other industrialized countries which are members of the Organization for Economic Cooperation and Development, where energy consumption per unit of GDP has always been roughly half that in the United States, the fall since 1970 has been more dramatic — more like 20 per cent than 17 per cent.

Two quite separate trends are apparent in the figures. First, all of the industrialized countries are using less energy for each dollar's worth of wealth that they generate internally. Second, within the total pattern of energy consumption, the importance of oil is steadily declining. All this is what would have been predicted from the elementary rules of supply and demand. The only surprise is that there has been such a noticeable change in a mere six years.

So does this mean that OPEC is now finished as a determinant of the price of oil and, by extension, of other forms of energy? That is not a reasonable calculation. Part of the reason why oil consumption has been depressed since the beginning of the decade is that economic activity has been at a low ebb, and part of the reason for that long depression was the sharp increase of the price of oil. OPEC's strength has during that period been contained by a kind of negative feedback which few industrialized economies have enjoyed. Ominously, with the improvement of economic activity between 1983 and 1984, there seems to have been a sharp increase of total energy consumption which restored energy consumption to the peak that it reached in 1979. (GDP had increased in the interval.) There is always a possibility that the full restoration of prosperity will have oil companies bumping up against the now much reduced level of production by the OPEC states, for oil is for technical reasons the most ready way of meeting marginal demands.

The moral in this for the oil consumers is that they must hasten plans for the use of other energy sources. Coal consumption has grown steadily, but only slowly, in the past decade, and there is plainly a limit to the speed with which supplies can be increased. Nuclear energy similarly lives on a long time-scale (that could and should be shortened). Energy conservation measures are similarly slow to take effect. But these and other devices are, if anything, more necessary now than at any time in the past decade. The unpalatable alternative is to see the price of oil increase yet again. □

Japanese science

Selected council to replace over-democratic body

Tokyo

THE newly selected Science Council of Japan is to go into action later this month. And selected, rather than elected, is the word, for the government has had its way and turned the often-troublesome freely-elected "parliament of scientists" into a select advisory body. In the process, nearly 80 per cent of its members have been replaced.

Since 1949, the Science Council of Japan has been advising the government on science policy and promoting and coordinating scientific research both nationally and internationally. Its activities have been directed by its 210-member general council and performed through standing committees and research liaison committees involving some 1,500 scientists.

Council members used to be elected in a postal ballot, held every three years, in which virtually all of Japan's 240,000 scientists were qualified to vote. The council was thus perhaps unique among nations in providing all scientists with the right to participate directly in choosing representatives to the highest level of government: the chairman of the council had an automatic right to membership of the Prime Minister's Council for Science and Technology. Numerous successes were scored by the council over the years and many of Japan's finest research facilities grew out of its recommendations including the Nobeyama radio telescope, the Photon Factory, the Institute of Molecular Science and the Polar Research Institute.

But the council ultimately fell prey to a peril for all "grass roots" democracies, the apathy of the "moderate majority". The council has always contained a "peace" lobby which irritated the government by its opposition to nuclear power, by action that successfully prohibited research on nuclear weapons, and by demanding parliamentary action to guarantee "freedom of research" rights of scientists. It was such people that campaigned most vigorously at election time, not a cheap process for although only the sending of promotional postcards was permitted, many candidates spent as much as a million yen (US\$4,000). Over the years they gained a larger voice in the council.

In return, the government tended to pay less attention to council advice and relied more on its own advisory bodies such as the Ministry of Education's own science council. And that meant that many voters, perceiving the council's power to have lessened, became indifferent to its activities and abandoned it to "left-wingers" whose interest was more

political than scientific, or that at least is the story the government would have people believe. The affairs of the council were costing a lot of money, which had to be found by the government. Supervising elections alone, with an electorate of nearly a quarter of a million, cost 100 million yen (US \$400,000 dollars).

After a few years of dispute with the government (see *Nature* 305, 361; 1983), the council is now completely reformed (or rendered subservient, depending upon the point of view). Representatives are not directly elected but are chosen at meetings of an electoral college (the Council Selection Management Committee) which is elected, together with candidates for council membership, by the major academic societies.

French science

Horizon freed from bonds

Paris

FRENCH science seems set for another good year and perhaps for three. When the budget of the French ministry of research came up for parliamentary approval at the end of June, the Prime Minister himself, M. Laurent Fabius, opened the debate.

He told the assembly that "when the question is research, the future of our country is at stake", and said that "the whole effort of this government is found on the triangle of research, training and investment". The fact that the opposition walked out is taken at the ministry as a positive sign for 1986, when the opposition seems likely, by all opinion polls, to be returned to power. Abstentions, or a vote against the budget targets, would have been much worse than a walk-out.

The divided opposition in France, led by M. Giscard d'Estaing, M. Jacques Chirac and M. Raymond Barre, can hardly bring itself to vote for anything that the present government has done, when the popularity of the President, M. François Mitterrand, is at a low ebb. But science and technology are widely thought to be among the government's successes. Thus the innovation director of a Paris company, who "cannot stand" the present government, admitted last week that the attitudes of French academics have now "completely changed". Previously, he said, academics liked to prove that they had no time for practical affairs. Now, they compete for industrial clients.

As for money, M. Fabius told the national assembly, "two periods have marked the development of science in

The new system, along with a rule preventing anyone serving more than three terms of three years each, has led to a dramatic shift in council membership (plus a huge reduction in expenses). Of the 210 members, 163 are new. In come many of the most famous names in Japanese science, as well as a large number of very active researchers who had earlier refused to be involved. The average age of council members rises a couple of years to nearly 63, perhaps because of the influx of emeritus professors, and so does the number from the Tokyo region, up to 64 per cent of the total. Tokyo University professors alone take 17 per cent of the places, with Kyoto next at 8 per cent. Women too have done a little better, if a change from just one representative to a mere three can be called an improvement.

There is no doubt that the new council contains an extremely distinguished group of scientists who will put science before politics. The test will be whether they pay attention to the wider implications of the expansion of science they will no doubt try to promote.

Alun Anderson

France. One was under President Mendès-France and one under General de Gaulle." There was a great decline after de Gaulle (beginning with President Pompidou) in which French research and development spending fell from 3 per cent of the gross national product to 1.7 per cent. Now it has been pulled back to 2.25 per cent, which marks a very strong growth since 1980. However, Fabius was careful in his choice of date: M. Mitterrand and his government came to power in 1981, but the science budget was already beginning to increase substantially in 1980 under President Giscard d'Estaing's science minister M. Pierre Aigrain. So science spending is not a party issue.

In the meantime, M. Fabius promised to give science "an absolute priority", backed M. Curien's proposal to raise the research budget by 4 per cent a year in real terms to 1988, and set his country's sights on reaching 3 per cent of gross national product by 1990-91. "No science policy is possible without continuity", Fabius said. M. Curien's "three year plan" will create 1,400 new scientific posts per year, will increase the number of student grants and "develop mobility".

Other themes raised by M. Fabius were the linkage of the research community to industry; a "primordial" commitment to scientific development of Europe as a whole and promotion on merit. In forging links with industry and in returning to an academic meritocracy, the present government may ironically have had its greatest success — and lost most of its ideological socialist support.

Robert Walgate

West German universities

Erosion of plans for change

Hamburg

NEARLY 40,000 students took part in a demonstration against the proposed amendments to German university regulations in Bonn during June, marking another crisis in discussions that have already dragged on for two years. The present government had commissioned a group of experts to review the existing regulations, the *Hochschulrahmengesetz* (HR6) and suggest improvements. These regulations were themselves a delayed response to the student unrest in the late 1960s, and had required four years of complex negotiation before coming into force in 1976 under the then Social Democrat/Liberal government.

One important feature of the earlier proposals was to shift the balance within university committees to a wider group, making it possible for professors to be outvoted by the combined representation of students, assistants and non-academic personnel when they are not unanimous among themselves. The plan to return to the former system, supported by the Education Minister Dorothee Wilms (Christian Democratic Party, CDU), is the main reason for the students' protest, although they also object to the proposed introduction of a two-tier system of studies and the relaxation of rules for industrial research within universities.

The amendments would allow particularly talented students to take additional courses while the majority would have to complete their studies after six semesters. Critics feel that this would divide graduates into two groups, one of which would pick up the good jobs while the other would have to compete for an ever-diminishing number of academic openings. And the planned relaxation of the regulations covering research paid for by other than official sources, usually by industry, has created intense mistrust.

Minister Wilms is becoming increasingly isolated, as the students are no longer alone in their protests. Even the West German Conference of University Rectors (WRK), once the prime mover of reform, has distanced itself from the present proposals, which it believes go too far. It is even being asked whether amendments are necessary after all. Meanwhile, the education minister is losing further ground: her hopes that the new regulations would be passed before the summer parliamentary recess have been crushed.

Even states governed by CDU have started to question the need for a change. The chief minister of Rheinland-Pfalz, Bernhard Vogel, has declared that the present regulations "are better than their reputation", on the grounds that they have enabled the universities to recover from the crisis of the 1970s. This reflects what most state governments are think-

ing.

The *Länder*, which would have to implement such new regulations as are eventually embodied in a new federal law, also feel that the change is unnecessary. Most have learned to live with the present law, with all its problems. This is exemplified by Bavaria, which has adopted a law to facilitate additional financing for research which is acceptable both to industry and the universities without having had to wait for changes in the federal law.

As time goes by, the opposition grows stronger. It is now uncertain whether the new proposals, even as amended, will be accepted by the *Bundestag* and the *Bundesrat* in the next parliamentary session. Speakers for the Social Democratic Party (SPD) have moreover declared that the plan of Dorothee Wilms to undermine the principle of the comprehensive universities (*Gesamthochschule*) is unacceptable, as is the proposed dissolution of the Study Reform Commission, a body concerned with the improvement of study courses. SPD even promises that the cancellation

of the new regulations would have high priority if it were to return to power. After its recent electoral successes (see *Nature* 6 June, p.450), SPD has become very self-confident.

Second only to the students, the loudest protests have come from the universities themselves. Having come to terms with the present law, they feel that politicians should concentrate more on the real problems: the steady erosion of financial support, the fact that 1.3 million students are enrolled on courses originally planned for 800,000, the rapidly increasing trend to unemployment among graduates and the exceptionally discouraging prospects for promotion within the university system, where the available professorships were distributed too readily during the years of expansion.

Despite all the criticism, Minister Wilms is insisting on her plans. They may represent the only significant project available to her, especially if the lifetime of the present government is limited. But her proposals are becoming less and less acceptable as the debate continues. Yet if the new proposals are not soon made law, little will remain of their original obviously reactionary character. **Jürgen Neffe**

AIDS

Blood test trials inconclusive

Washington

THE first actual performance data on the acquired immune deficiency syndrome (AIDS) blood test are now in and they are a mixed bag. The test became available to blood banks in the United States in March following an all-out effort by the Public Health Service (PHS) and five contractor companies to meet a six-month deadline imposed a year ago by Secretary of Health and Human Services Margaret Heckler.

The performance data suggest that at least some of the concern voiced by critics of the rush effort have still not been adequately addressed. Of the 593,831 units of blood tested and reported to PHS during the four weeks beginning 22 April, 0.89 per cent tested positive in the first instance for AIDS antibody. That rate is not inconsistent with earlier estimates of the prevalence of AIDS antibody in the general population. But only 0.25 per cent of the blood samples were "repeatedly reactive" which, according to the PHS definition, means that they test positive twice in a row by the test or two times out of three if tested three times. The tests now in use all use enzyme-linked immunosorbent assays (ELISA) to detect the presence of antibody to the AIDS virus, HTLV-III/LAV. The new data cover about 70 per cent of all blood collected in the United States during that four-week period.

Although the data say nothing about the false positive rate, one of the major concerns at the outset, the large number of samples that tested positive initially but

negative when rechecked, is a worry. Test kits produced by at least two of the five companies, Abbott and Electronucleonics, showed wide variations of activity at first; Electronucleonics even had to recall from blood banks one early batch of kits on this score. Although improved quality control has done much to correct the problems, some variability apparently remains.

Meanwhile, at least some of the false positive reactions have been explained. People with certain HLA antibodies appear to react consistently positive to at least one of the tests on the market. It may be that because the test kits use virus antigen grown in human cell lines, some of the HLA antigens found on the human cell membranes are picked up in the purification of its virus protein. This problem may be eliminated when test kits derived from genetically-engineering virus antigen (grown in *Escherichia coli* rather than human cell lines) become available. These are being developed by Genentech and Centocor; clinical trials are expected to begin within two months.

So far, no data are available on how the ELISA test kits' performance compares with independent (and generally more reliable and more expensive and difficult) tests such as the Western blot. Some initial data will be presented at a conference at the National Institutes of Health on 31 July. These results should provide the first accurate reading on the false positive problem. **Stephen Budiansky**

Eureka

France puts flesh on bones

Paris

EUREKA, the French-inspired programme to unite European high-technology industry behind some concrete, profitable and advanced joint enterprises, has a public shopping list at last. Last week CESTA, the Centre d'Etudes des Systèmes et des Technologies Avancées, a public agency with an office in the very courtyard of the French ministry of research, produced a 70-page document listing five "priority action programmes" for Eureka. It is the product of two and a half months of intensive effort by the small and now somewhat fatigued interministerial Eureka group within the French government.

Until the European conference of research and foreign ministers in Paris later this month, the CESTA proposals are the nearest to a bible of exactly what Eureka is — or could be. Only "some" of the proposals are the result of recent shuttle diplomacy; the rest spring from unilateral French imagination.

The CESTA report defines five priorities: computers, networks and communications, robotics, biotechnology, and materials. And in each, it concludes with specific projects, timetables and potential European partners.

Computers dominate the proposals. In a programme dubbed "Euromatic", the Eureka group suggests that Europe should develop by 1992 a "super-Cray", capable of the 30 GigaFlops (floating-point operations) per second "required in the design of complex systems". It should be vectorial, use gallium arsenide chips (as yet unperfected technology), and could link the French companies Bull (once Honeywell-Bull) and Thomson with Siemens in West Germany. Although the market for such machines may be small, they are becoming so "essential" in certain key applications that Europe should develop a strategic independence in the technology from the present US and, shortly, Japanese suppliers.

Also under "Euromatic", the French group identifies a need to study "highly parallel" computer architectures aimed at a machine of 10 GigaFlops by 1992, via a demonstration machine 100 times less powerful by 1988. This would involve collaboration between Bull, Thomson and the German computer company GMD, which would "form the core of a broader European team including other partners (for example Britain, Italy)".

Other Euromatic projects include the development of high-capacity mass memory, starting with "giant" magnetic disks and moving on to optical disks (Bull, plus BASF and Siemens of West Germany); a software engineering centre (five companies in France, four in West Germany plus Aachen University, Philips in the Netherlands and ICL, Logica and Scicon

of Britain are suggested); artificial intelligence applied to "four precise projects" (nine companies and one university in France, four companies in Britain, Olivetti of Italy, Norsk Data of Norway and Philips and the University of Amsterdam could be involved); and there would be work on synchronous architectures, dedicated circuits and symbol processors.

The prospectus also lists projects on expert systems, multilingual access to databases, the management of large-scale industrial processes, a European microcomputer "paving the way to advances in integrated circuitry and the image processing industry, now a US monopoly", a 64-megabit dynamic memory and a European development unit for gallium arsenide technology and custom circuits.

Euromatic is by far the most specific of the five "priority actions". In robotics ("Eurobot"), France proposes the development of intelligent semi-autonomous robots for repair and maintenance in hostile environments (such as nuclear reactors), an entirely automatic agricultural tractor (Renault, Fiat and Saab could be interested), flexible-production factories (using computer-automated design and manufacture) and carbondioxide, carbonmonoxide, excimer and free-electron lasers as machining tools.

"Eurobio" involves the production of new crops and varieties for the seed industry, and the development of crops "as a means of transforming industrial processes"; and the research and development of systems for the control and regulation of biochemical processes (including biochemical reactors and novel means of drug delivery). The objective in the seed industry would be to give Europe "a leading position" on the world seed market, estimated to be worth some \$10,000–12,000 million, and some two dozen European companies, institutes and universities are identified as potential partners.

Add to these programmes "Eurocom" (communications) and "Euromat" (the development of a ceramic turbine) and you have a list of most of the high technology challenges facing not just European but all developed-world industry. So what is special about Eureka? First, the sense of urgency. Second, the fact that even the ideological French have discovered pragmatism; they say here that "nothing is fixed" about Eureka except that some practical actions should be under way by the end of this year. The key is probably government backing. "We don't expect governments to be coming with their checkbooks" to the Eureka conference later this month, they say, "but we do want to define the structure of financing". Which means it is time to say who will pay, and how.

Robert Walgate

UNESCO

Reforms now under way

Washington

A SERIOUS attempt is being made by the United Nations Educational, Scientific and Cultural Organization (UNESCO) to answer criticisms by Western nations. The executive board, meeting in Paris, has urged strengthening UNESCO's scientific and environmental activities, which are generally well thought of, but also put limits on more controversial programmes such as "Communication in the service of man". US officials seem pleasantly surprised by reports of the meeting but stress that no immediate decision to rejoin is on the cards.

Decisions taken at the marathon six-week meeting in Paris are not binding on the organization; they will however be put to the UNESCO general assembly next October in Sofia, Bulgaria, where all 260 member countries will be represented. The most pressing task has been to plan a 1986–87 biennium budget that is 25 per cent lower than the previous biennium, due to the withdrawal of the United States. Western nations (particularly Britain, which has also threatened to withdraw unless there are improvements) wanted to see the selective elimination of programmes that were judged to be either wasteful or opposed to Western interests; the Soviet Union, on the other hand, wanted to see a 25 per cent reduction across the board. So far, the West seems to have had its way.

The communications programme, for example, has been made uncontroversial by strictly limiting its more theoretical aspects, while devoting most of the funds for this topic into training of journalists. The Intergovernmental Programme on Man and the Biosphere, the International Geological Correlation Programme and the International Hydrological Programme, on the other hand, have been recommended for strengthening.

There has also been a reduction in the number of subprogrammes in the draft budget (another Western bugbear), and the whole plan has been presented in a new format that breaks with convention by being relatively easy to understand. Cost-benefit analysis is used explicitly, apparently for the first time.

The executive board has essentially ended up with a zero growth budget, with all activities beyond those that can be paid for after the 25 per cent budget reduction placed in a special category. The board suggests that similar planning should be adopted if Great Britain and Singapore do withdraw after the next assembly, as threatened. The changes so far at UNESCO, though pleasing to Western eyes, are not considered likely to make Britain change its mind.

Tim Beardsley

French telecommunications

National data network crashes

Paris

THE technical revolution in the French telecommunications system has been temporarily stopped in its tracks.

After being transformed from rustic to semidigitized in ten years, the change has been so great that it has entered the language. To be fashionable in France these days is to be "*cablé*", or "wired-up". Last year, it was "*branché*" ("plugged-in"), or even better the transposed "*chébran*".

But now and for two months, Transpac, the French and the world's most-used packet-switched data network, will be out

of bounds to individual users. Last week, private users so overloaded the system with their messages and consultations of electronic newspapers now on full-text database that the whole network crashed.

The cause is a bug in Transpac software for assembling and disassembling data packets, the groups of a few characters of a message which are given heads, tails and an address and then sent independently through the network so as to make efficient use of the gaps in other messages. "Packet assembler-disassemblers" (PADs) using this software are distributed throughout Transpac, and are designed to shut down when overloaded. At the highest levels of use reached last week, however, one PAD shut-down caused such an increase of traffic through others that they also shut down, leading to a chain-reaction which closed the whole of Transpac almost instantly.

This may have irritated the 800,000 or so home-users who now have access to Transpac through their free "Minitel" terminals, distributed by the telecommunications authority, PTT, to replace printed telephone directories. But it was disastrous for the banks, airlines and other companies with high data traffic that now rely on Transpac for business. Hence the decision by PTT to unplug the poor *cablé* public, until September, by when the Transpac bug should be sorted out.

Meanwhile the free distribution of Minitel (simple screens and keyboards) has now been suspended, but earlier projections were that the number of *Minitelistes* would more than double to 1.7 million this year, and perhaps reach 3 million by 1987. Transpac could cope with this if it were not that *Minitelistes* are not behaving as expected.

The typical "*tapeur*" at the keyboard does indeed consult the few hundred databases available — that much was expected — but, at least until the great crash, he or she spends much more time sending electronic messages to other users through systems such as "Missive", an electronic mailbox supported by PTT. And a little arithmetic shows that while 3 million times a few hundred databases is a large number of connections, 3 million squared is huge, probably too huge for even Transpac to cope with it.

That is why there is now some concern that the apparently logical French approach to create a single giant data network may not be so logical after all. For while the roads and rail seem capable, on the whole, of carrying both goods and people, it may turn out that personal data habits and the needs of industry (transferring large files with infrequent calls on a few trunk routes) are so different that quite different networks and strategies will be needed.

Robert Walgate

University of Geneva

Karl Illmensee resigns

DR Karl Illmensee, professor of biology at the University of Geneva, has resigned his post with effect from 30 September 1987 the date on which his present contract would normally expire. Illmensee's decision was submitted some days before a meeting of the professors of the University of Geneva called for 1 July at which the faculty was to have decided whether or no his appointment should be extended. The meeting would have had before it a report on the events of the past two years, during which the validity of some of Illmensee's published work has been questioned.

This latest development is probably a consequence of the report's recommendation that Illmensee's appointment should not be renewed. The report was prepared by a faculty commission appointed last February under the chairmanship of Professor R. Epstein, of the University of Geneva. Professor Illmensee had already been asked to provide a document giving his comments on the events of recent years, which is analysed in the report prepared for last week's faculty meeting. In the event, the matter was not discussed.

The Epstein report says that the quality of Illmensee as a teacher is not disputed but that there are reservations about his diffidence in sharing his techniques with collaborators. Without "wishing to give too much weight to the matter", the report also finds that Illmensee had overstated his wife's qualifications for her work as an assistant in his laboratory. Neither of these matters was taken up by the international commission looking into the more serious charges against Illmensee which reported in February last year.

Of the charges that some of Illmensee's published work is false, the report says (in respect of a series of experiments in which parthenogenetic mice were born) that there is no objective basis for proving Illmensee's observations wrong, but that it is a fair complaint that he had made no attempt to repeat the experiments.

The faculty commission makes the same complaint about Illmensee's failure to repeat the experiments involving the transplantation of nuclei into embryonic cells. Dealing with Professor Illmensee's unpublished defence, examined by the Epstein commission, the report says it does not accept his contention that he lacked the time and the means to repeat the experiments.

The commission explains its unwillingness to follow the report of the international commission by saying that that report was more damaging than its failure to find proof of deception would have suggested. The Epstein report also says that repetition of the disputed experiment was a key recommendation.

Charity for MRC

A MAJOR new institute is to be set up within the University of Oxford to apply molecular biology to medicine. The British Medical Research Council (MRC), which has been the prime mover in founding the institute for Professor David Weatherall but which has not had the cash to do so, has finally enlisted the aid of the Wolfson Foundation to the tune of £1 million. Building should take two years.

The object of the provisionally named Molecular Medicine Institute, which will be on the site of the John Radcliffe Hospital in Headington on the outskirts of Oxford, is "to create a critical mass of good science and scientists within an essentially clinical environment", says Weatherall. Each of the five or six groups within the institute will be fully integrated into the clinical school and will need to be self-sufficient in research money. MRC and Wolfson have provided the capital costs, with help from the university and the Cephalosporin Trust.

For the Wolfson Foundation, this is the first formal collaboration with any research council and the case, says Wolfson director Dr J. Black, was simply considered on its merits, which were considerable, according to Sir James Gowans, secretary to MRC. "Our collaboration is an example of the council not necessarily being resource-led. When something is important enough that it has to be done and government policy means that the resources are not available, it is a question of finding help."

Neither partner will agree that Wolfson bailed out a previously committed MRC, but Black says there has been no discussion of a continued collaboration between the two and any new request would be treated on its merits. With the present financial state of the research councils, it seems likely that they will nevertheless look more eagerly at the £7 million Wolfson dispenses each year, mainly for the capital cost of constructing new laboratories.

Peter Newmark

Swedish security

Too much free information?

Stockholm

THE Soviet Union has denied using the international interlibrary loan system to obtain sensitive defence-related information from Sweden. A spokesperson of the official Soviet news agency APN pointed out last week that, if the Soviet Union did indeed require such information, it could acquire it by a "clearer and safer method", using satellite photographs. The Swedish defence staff has, however, launched a special inquiry into possible leaks through the interlibrary loan service.

International loans from Sweden are handled by Lund University Library, one of the two Swedish copyright deposit libraries that receive a copy of every work published in Sweden. (The other is the Royal Library in Stockholm.) The Soviet Union borrows between 750 and 1,300 works from Sweden each year, mostly in the humanities. So far, only a handful of "sensitive" items have been requested, all so far from "open" unclassified literature. To refuse to send such items would run counter to Sweden's policy of open access to information, and would require the special intervention of the government. This, according to Nils Palmberg, the Lund librarian in charge of the loan service, is hardly likely at the present moment.

According to Palmberg, Lund was first

alerted to the possibility of the loan service being used for non-academic ends some three years ago, when an East German naval library requested a copy of *Marinnytt* (Marine News). This is in fact readily available from all Swedish embassies and cultural institutes, and the main puzzle about the request was why the East German navy should bother to use the loan service when they could simply have picked up a copy in East Berlin. The Lund librarians duly forwarded the copy, but felt it worthwhile to inform the Swedish defence staff and to keep a watch for further puzzling requests.

In autumn 1984, they received a request from the Soviet Union for details of naval harbours in Norrland (North Sweden) and of a hydrographical survey of the same region. Later came a request about an area of central Skania (southern Sweden) where there are several military installations. According to Bertil Lagerwall, chief press spokesman of the defence staff, the material requested in the latter case included maps, photographs and also details of new building works in the area, all of which would be of considerable interest to anyone planning an airborne invasion.

According to Palmberg, the Lund library has worked out an arrangement with the defence staff about such requests.

Lund will forward the material to the library filing the request (in the case of the Soviet Union, the Lenin Library in Moscow), and will then notify the defence staff of what has been sent. The rationale here seems to be that, since the defence staff is notified only after the material has been sent, the arrangement does not compromise Sweden's commitment to the free flow of information. There does not seem to be any overt international agreement that requests for the transfer of information should be a matter of confidence between the parties concerned, and the Swedes presumably feel that, if the motive behind the request is innocent, the person or organization filing it would have no valid objection to a third party (in this case the defence staff) being informed.

For whatever reason, both Lund and the defence staff kept quiet for several months about the agreement, which was worked out last February. It was first revealed by the *Sydsvenska Dagbladet* two weeks ago and evoked considerable public comment and concern. According to defence spokesman Lagerwall, however, the Swedish government's commitment is clear: "We cannot and will not censor open literature", he declares. **Vera Rich**

Hans-Otto Wüster

THE European thermonuclear fusion project suffered a serious blow at the end of last month, with the unexpected death of Dr Hans-Otto Wüster, the director of the JET (Joint European Torus) project. Dr Wüster (58) was a West German theoretical physicist with a PhD from the University of Köln, where he worked for seven years at the Institute of Theoretical Physics. Thereafter, he spent successive spells



at the Deutsches Elektronen-Synchrotron (DESY) at Hamburg and at CERN, Geneva, where he was deputy-director between 1971 and 1975.

Dr Wüster was appointed director of JET in 1978. During his time, the torus was commissioned successfully. Much of Dr Wüster's contribution during his spell as director was to ensure political support for JET. But the laboratory says that he will also be much missed for his strong but sensitive style of management. □

Giotto now on target for Halley's comet

THE Giotto spaceprobe, the European Space Agency's contribution to the fleet of six craft aimed at Comet Halley, was successfully launched by an Ariane 1 launcher from Kourou in French Guiana last week. The three-stage launcher placed the satellite in a geostationary transfer orbit, from which an on-board motor injected the craft into a heliocentric trajectory aimed at an encounter with Halley on 13 March next year.

One important aim of the mission is to enter the coma of the comet and, using a four-colour camera, obtain images of the nucleus, possible only from within the coma. The best resolution hoped for is 50 metres. The composition of the coma itself will be sampled by neutral and ion mass spectrometers and dust detectors, while

the electromagnetic environment will be probed by magnetometers and plasma analysers. The spacecraft is not expected to survive beyond its closest approach to the nucleus of less than 500 kilometres due to the effect of dust impacts — its relative speed is expected to be 68 kilometres per second.

These experiments will to some extent be complemented by those aboard the five other spacecraft that should encounter Halley at about the same time (see table). Giotto's instruments will be switched on in September or October. In particular, the plasma analysers will be used to sample the solar wind at the time of the encounter of the International Comet Explorer with the comet Giacobini-Zinner on 11 September.

Philip Campbell

Probes to Halley

Mission	Launch date	Encounter date	Closest approach	Nationality
Giotto	2 July 1985	13 Mar. 1986	500 km	Europe
Vega-1	15 Dec. 1984	6 Mar. 1986	10,000 km	USSR
Vega-2	21 Dec. 1984	9 Mar. 1986	10,000 km	USSR
Sakigake	8 Jan. 1985	11 Mar. 1986	7,000,000 km	Japan
Planet-A	14 Aug. 1985	8 Mar. 1986	200,000 km	Japan
International Comet Explorer	22 Dec. 1983*	28 Mar. 1986	32,000,000 km	USA/Europe

*Originally International Sun Earth Explorer-3, currently set to encounter Comet Giacobini-Zinner followed by Halley.

NERC's infrastructure defended

SIR—I wish to respond to the letter from J.S. McPherson (*Nature* 13 June, p.536) commenting inaccurately on the role of Natural Environment Research Council (NERC) HQ and NERC Scientific Services (NSS). The staff numbers he quotes are correct, but to say that "... NSS is largely administrative in composition" is wrong and grossly unfair to the 90 per cent of NSS staff who are scientists and technologists providing support for the research carried out by both institute and NERC-supported university scientists. Most of NERC's administrative staff are in the institutes.

A more than superficial perusal of NERC's annual reports for the four years 1980/81–1983/84 would reveal that in that period the "quality" of computing provided to institutes has increased by a factor of four, new and sophisticated marine

equipment has been brought into service, an image analysis facility has been brought into use (and incidentally attained a reputation sufficient to attract a Principal Investigator award from the National Aeronautics and Space Administration) and an instrumented aircraft made available for NERC-supported science. And all this has been achieved with staffing that is sensibly constant over the period.

Turning to the question of commissioned research, NSS has not, as a matter of policy, sought to sell its services in the market place. What is clear from the annual reports is that many of the contracts obtained by the institutes would not have been possible without the computing, the ships, the cartography and other support services provided by NSS and administrative services of HQ. It is regrettable that Mr McPherson does not

seem to have the text of NERC's annual report which describes the vital support role in which NSS staff are engaged.

Mr McPherson's letter contains other errors. He says that it is sad that 1,000 scientific jobs are to disappear. The NERC corporate plan published last February indicates that 900 (not 1,000) posts may be lost over the next five years. Staff reductions will be in all categories—scientific, technical and administrative—with the aim of maximizing scientific output in relation to available income. NERC would prefer not to have to reduce staff numbers but it has to be realistic, live within its means and provide its scientists with the equipment and support services which they need to be effective.

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The science of sea-dumping

SIR—We wish to comment on the independent review of the practice of dumping radionuclides in the oceans, commissioned by the UK Department of the Environment, carried out by a group under F.G.T. Holliday and published last year¹. The terms of reference were to "review the scientific evidence, including the environmental implications, relevant to the safety of dumping radioactive waste at the designated North Atlantic site". The report gives a detailed description of the history of dumping and of the structure and operation of the model developed by the National Radiological Protection Board (NRPB) and the Ministry of Agriculture, Fisheries and Food (MAFF)^{2,3} which is used to assess the environmental effects of disposal.

Scientific modelling should clearly specify the model in terms of the underlying theoretical assumptions, and should indicate specific tests by which the model can be validated.

In the Holliday review, we find little discussion of the theory and none of experimental validation. The review notes that "the modelling exercise is complex and involves hundreds of man-hours of work which it has been impossible to check", but concludes that "the science being undertaken in the United Kingdom is of the highest quality. The results are published in the open literature and are openly discussed at international meetings, and this provides some safeguards on the reliability of the work."

Thus it is proposed that the ultimate guarantor of the quality of science being undertaken lies in some form of international peer review. We do not wish to comment on this process as it affects the MAFF/NRPB model as a whole but the part which concerns biological pathways.

The biological pathways sub-model is a crucial component of the whole because it concerns the potential concentration of hazardous materials which are otherwise dispersed in the ocean. In the MAFF/NRPB model the trophic level concept⁴ is used as the basis for calculating the concentration of radioactivity in marine life. Yet after the work of the International Biological Programme (1964–74) it became clear that although trophic level one (green plants) could be identified and trophic level two (herbivores) could be found in terrestrial systems, the remaining "trophic levels" could not be adequately identified⁵. This made the concept untestable and therefore suspect. Current concern about the inapplicability of the trophic level concept to aquatic ecosystems is shown by Ulanowicz and Platt⁶.

In the MAFF/NRPB model, it is assumed that the main food chain can be modelled by six trophic levels plus humans. Misleading conclusions can arise from this view that the food chain is limited in length. Materials cycle through the food web by organisms investing faeces and carcasses, creating food chains which are in trophic level terms indefinitely long^{7,8}.

Furthermore, science is surely about testing models, not simply asserting them, however authoritatively. The Holliday review illustrates several *predictions* about the effects of sea-dumping, but does not contain a single experimental result or reference. It is claimed that the model has been "adjusted" or "forced" to reproduce certain physical features of ocean dispersal, though this in itself does not imply predictive value. The final result of extremely long-range forecasting, involving release from dumped containers, oceanic dispersal and passage through food

chains, is a predicted radiation dose to humans over the next 100,000 years (peaking at 100 years). A minimum requirement for the credibility of such predictions is experimental validation in some simpler case. For example, earlier sea-dumping (1950–63) was carried out, without containment, in the English Channel; there has been the possibility of 30 years' monitoring of this more accessible release for comparison with predictions. Furthermore, much data have been collected on contamination of the Irish Sea, which could also be compared with model predictions. Are there no results of validation studies? What would the Committee on Safety in Medicines think of predictions not backed up by clinical trials?

The Holliday report does prudently recommend that sea-dumping not be resumed "until the current international reviews and the comparison of sea-dumping with land-based alternatives have been completed". It is essential that such reviews should address the two issues—the theoretical basis of the model and its experimental validation—that are absent in the current report.

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Measuring the unmeasurable

After more than a century, the reality of Maxwell's displacement current has been demonstrated by direct measurement. So, too, but less conclusively, has been the Aharonov/Bohm effect.

ONE of the big surprises of Maxwell's theory of electromagnetism was his introduction of the concept of displacement current. By Maxwell's time, it was generally understood that an electrical current in a conductor would generate a magnetic field (Ampère's law applies). In modern language, the relationship is that the curl of $\mathbf{H}$, the magnetic field strength, is equal to the current, or that $\nabla \times \mathbf{H} = \mathbf{J}$, where $\mathbf{H}$ is the field and $\mathbf{J}$ the current, both of them vector quantities. Maxwell's innovation was to add to the right-hand side of the equation the rate of change with time of the electrical displacement, $\mathbf{D}$, related to the electric field strength by $\mathbf{D} = \epsilon \mathbf{E}$, where ϵ is the scalar permittivity of the material, itself related to the dielectric constant by a numerical factor depending only on the choice of units. Now the displacement current has been measured for what appears to be the first time.

None of this implies that the reality of the displacement current has been in limbo in the interval. Indeed, were it not for the displacement current, it would not be possible to deduce from Maxwell's equations that electromagnetic waves have the properties of light. Maxwell was well aware of the difficulty he was creating. Indeed, D.F. Bartlett and T.R. Corle from the University of Colorado at Boulder, who have now measured a displacement current directly (*Phys. Rev. Lett.* **55**, 59; 1985), quote him as describing as one of the "chief peculiarities" of his thesis "that the time derivative of the electric displacement must be taken into account".

The principle of the measurement is a good deal simpler than the practice. The obvious first step is to create a reasonable source of displacement current. What could be simpler than the steady increase of electrical displacement between the plates of a capacitor during the process of being charged, most simply by connecting its plates to a physical electric current?

Bartlett and Corle point out two obvious snags. First, the voltage would quickly reach the point at which there would be a spark between the capacitor plates. Second, since the obvious way of measuring the displacement current is to measure the associated magnetic field, the feasibility of the measurement is determined by the relative magnitude of the field and the sensitivity of available measuring devices. Bartlett and Corle point out that for realistic capacitors, the

expected magnitude of the magnetic field will be measured in micro-gauss.

From there, the design of the measurement is determined. The ideal of a direct-current measurement is unattainable, but a low-frequency current will do instead provided that the response-time of the ultimate measuring device is faster than the oscillation. The other requirement, that it should be possible to measure magnetic fields in the micro-gauss region, points uniquely to a SQUID (for superconducting quantum interference detector). And that, inevitably, points to the source of the complexity of the measurement, the need to contain the equipment in liquid helium.

Bartlett and Corle use a capacitor consisting of a pair of circular plates 7.6 cm in diameter and separated by 1.22 cm. The magnetic field between them is measured by a coil 1.5 mm in diameter encased in a stainless steel tube which, at liquid helium temperatures, acts as a shield against stray electric fields. The SQUID itself, based on a Josephson junction, is enclosed in a superconducting lead shield. If the arrangement were strictly symmetrical, the magnetic field induced by the displacement current, at some given phase of the driving current, would be represented by concentric circular lines of force with the field strength increasing linearly from the centre to the edge of the plates and then decreasing inversely with distance from the axis of symmetry.

At least to a first approximation, the geometry is exactly like that of the magnetic field associated with a current in a linear conductor except that, now, the current is displacement current and distributed across the whole area of the plates.

The complications are horrendous. Magnetic fields from the cables carrying the current are an obvious nuisance, if only because the measurement must be made inside a liquid-helium Dewar vessel and also inside a shield against stray magnetic fields from elsewhere. (The shield is a spherical copper vessel coated on the inside by superconducting lead/tin solder and filled with helium.) The leads carrying current to the plates are bent so that their final separate approach to the capacitor is along the axis of the arrangement, and also that the magnetic field generated by the power supply can be measured simply by rotating the measuring coil through 90 degrees. The use of an alternating power supply has the advan-

tage of allowing the phase of the oscillation of the displacement current to be determined directly.

The only surprise is the precision of the result. A plot of the field strength against distance from the centre of the plates is indeed a straight line, whose slope is determined by the small scatter of points to within three parts in 100. The measured slope differs from that calculated from the dimensions of the capacitor and the other parameters of the measurement by less than five per cent, possibly explained by the intervention of a steel tube between the two plates.

Not all physical measurements of this kind are so unsurprising, demonstrations as it were of the ingenuity of which physicists are capable. A few weeks ago (*Phys. Rev. Lett.* **54**, 2469; 1985), Giorgio Matteucci and Guilo Pozzi from the University of Bologna reported a measurement designed to verify the reality of the Aharonov/Bohm effect, the quantum phenomenon by means of which the phase of the wave function describing a particle (such as an electron) should be affected by the electromagnetic potential of an electromagnetic field even when the particles are not subjected to physical forces.

One of the first demonstrations of the effect, in which Matteucci and Pozzi also had a hand, consists in arranging that a beam of electrons will travel on either side of a superconducting tube with a magnetic field along its axis in such a way that magnetic forces do not affect the electrons. Although the apparent physical tracks of the electrons are unchanged, their phases are altered by the presence of the confined magnetic field, and interference fringes produced as a result.

What Matteucci and Pozzi have now done is to repeat the experiment with a thin platinum wire coated on one side only with a thin layer of aluminium. Because of the contact potential between the two metals, the electrons see the wire as if it were an unsymmetrical distribution of electric charge, but because the wire is thin, there is no effective force on the electrons passing on one side or the other. But yet again, because in this case the electrostatic potential is different on one side of the wire from the other, the phases change and electron interference fringes are produced. Indeed, the pattern of the fringes can be changed as the wire is rotated about its axis which will seem yet another *tour de force*.

John Maddox

Astronomy

An extraordinary gravitational lens and the redshift debate

from M. G. Edmunds

ONCE in a while a remarkable astronomical object is found in a fairly routine observing programme. Just such an object turned up last September during a galaxy redshift survey by the Harvard-Smithsonian Center for Astrophysics. The first published set of observations (Huchra, J. *et al.* *Astr. J.* **90**, 691; 1985) shows that right in the centre of an unremarkable redshift ($z=0.04$) spiral galaxy there sits the image of a high redshift ($z=1.7$) quasar.

There is no doubt that the quasar is of high redshift — the spectrum is almost a textbook example, showing strong hydrogen Lyman α and carbon-ion emission lines. The brightness and size of the galaxy (known by its positional coordinates as 2237+0305) are perfectly consistent with its low redshift. Yet the quasar is within about one-third of an arc of the centre, as judged on images recorded by a charged couple device solid-state detector when the terrestrial atmospheric seeing conditions were smearing point images to about two seconds of arc in size.

It is fascinating to speculate how the debate over the origin of quasar redshifts would have been affected if this object had been discovered twenty years ago, but it is unlikely that current theories of the non-cosmological origin for redshifts will have to be resurrected. Many cases are known of bright nuclei in galaxies that show quasar-like spectra (the so-called Seyfert galaxies) but whose redshifts (and by implication, distance) are the same as the host galaxy. The new object can probably be interpreted as a distant quasar, the image of which is brought towards us by the gravitational field of the nearby galaxy acting as a lens. The detection of the first gravitational lens (other than the classic deflection of light by the Sun) was reported in *Nature* in 1979 although they had been predicted by Einstein and championed by Zwicky in the 1930s. Five good cases of gravitational lensing of distant quasars are already known (for example, Turner, E.J., Ostriker, J.P. & Gott, J.R. *Astrophys. J.* **284**, 1; 1984), but these differ from 2237+0305 in that the galaxies causing the lensing are much more distant and they produce at least double quasar images.

The fun really starts when an attempt is made to estimate how likely it is that we should be able to find a system like 2237+0305. The assessment of *a posteriori* probabilities for a single object is, of course, a very subjective and uncertain task. Nevertheless, it is clear that this is a very unusual system. The chance of find-

ing such a bright quasar (approximately 17th blue magnitude) within an arc second of the centre of galaxies that have been spectroscopically surveyed would be only about one in ten thousand but, as with a conventional lens, the gravitational lens can not only bend light but also brighten the image. The difficulty, as pointed out by Huchra *et al.*, lies in the fairly large amplification factor that probably has to be invoked to increase the chance of finding such an object to, say, the few per cent level. If the quasar is not amplified, its intrinsic brightness (as implied by its redshift) must be unusually large.

Huchra *et al.* suggest that an amplification factor of 40 would give a reasonable probability of detection, and they claim that they can construct a lens model in which the required mass-to-light ratio of the galaxy is not unreasonably high (21 in solar units). But they neglect two factors in their revised probability estimate. The first is to fold into the estimate the probability of having a lens configuration that will produce such a high amplification; the configuration cannot be particularly common, since only very restricted conditions give high light amplification. The second problem is the effect of absorption by in-

terstellar matter in the galaxy through which the quasar is seen. Huchra *et al.* note that the optical continuum spectrum of the quasar appears to be highly reddened, showing a power law dependence on frequency of $\nu^{-3.5}$, compared with a typical $\nu^{-0.6}$ for most quasars. While it is possible that the object has an intrinsically steep spectrum, it is not difficult to show that the change in continuum slope from a typical value implies three magnitudes of extinction and hence requires yet another factor of 20 in amplification of the background object.

These two problems seem to make the detection probability dwindle too much for comfort, but it would be wise to wait for more detailed lens models and further observations before passing judgement. Higher spectral resolution observations reveal whether the quasar shows any sign of interstellar absorption features, such as calcium H and K lines and the sodium D line, which would give an independent estimate of extinction. Observations at deeper and higher spatial resolution, which will require seeing conditions of about half an arc second, may resolve the central image into subsidiary images which would allow better modelling of the lensing. The lensing explanation would, of course, be established were spectroscopic evidence that each of the split components had the same redshift to become available — an excellent project for the Space Telescope when it is launched. □

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Protein structure

A new twist for hairpin turns

from Jane S. Richardson

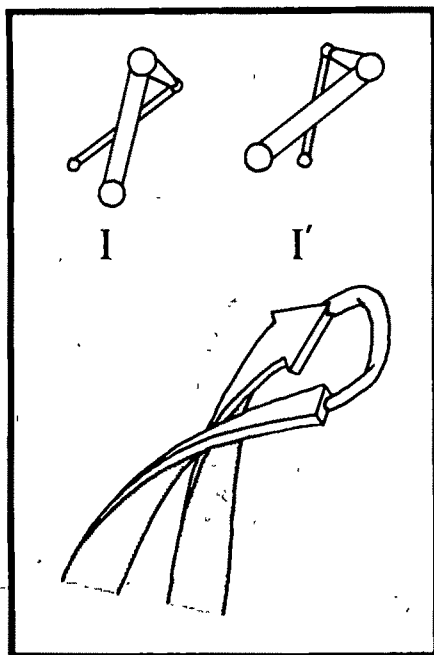
In protein conformation, the tight turn^{1,2} is considered the classic, neat solution for connecting two antiparallel strands of beta-sheet. However, in the course of surveying and cataloguing short beta-hairpin connections (see page 170 of this issue³), Lynn Sibanda and Janet Thornton have unexpectedly found that this apparently obvious combination is not so well matched after all.

Beginning with the chiral alpha-carbons of natural L-amino acids, conformational features at all levels of protein structure show strong handedness preferences such as the right-handed alpha-helix, the right-handed twist of beta-sheet (see figure and ref. 4), and the right-handedness of beta-alpha-beta crossover connections^{5,6}. Tight turns also have a handedness, as they are not really planar as often drawn but have a pronounced dihedral angle defined by the four successive alpha-carbons. In the most common turn, termed Type I, that dihedral angle is approximately -45° (ref. 7). There is a mirror-image version of the

Type I turn (called Type I') which has a $+45^\circ$ dihedral angle, but it is very rare because it puts the side-chain beta-carbons uncomfortably close to the backbone and so only works well with glycine.

Sibanda and Thornton³ now show conformational preferences for each size category of beta-hairpins. For the shortest hairpins (with only two residues connecting the last hydrogen-bonded pair in the beta-strands) they find only 4 Type I turns but 15 Type I'. This is quite startling, as it reverses the overall occurrence statistics by a factor of about 40. The unusual circumstance responsible for that reversal is presumably a strong interaction between the twist of the beta-sheet and the twist of the turn. Those twists match perfectly for Type I' but fight each other for Type I, as illustrated in the figure.

One can imagine this built-in conflict as resolvable in two distinct ways during the folding of a real protein structure. If a beta-hairpin initially formed with its turn in the standard Type I geometry, the beta-



sheet twist would put a torque on the turn. In some cases the outcome could be breakage of the one or two hydrogen bonds closest to the turn, usually forming a kink in that part of the hairpin with the result that it would be classified by Sibanda and Thornton as a four-residue rather than a two-residue beta-hairpin. This outcome is more likely when the turn side chains are bulky, and is probably the only way to accommodate a proline. In other cases the torque might produce a shift of ϕ and ψ conformational angles over to values suitable for a different turn type. Types I and I' probably do not interconvert, as their ϕ and ψ angles differ by an average of 120° . However, there is another mirror-image pair of turn types available called II and II'. Type I could probably be converted to II' while Type II could switch to I', greatly improving the match to the beta-sheet twist in both cases. This sort of conversion should be much easier if one or both turn residues were glycine, and indeed 21 of the 25 short Type I' or Type II' beta-hairpins were found to contain at least one glycine.

The ubiquity of alpha-helices in proteins partly results from the fact that the local and long-range conformational preferences coincide. For beta-hairpins, however, we are now led to imagine the protein as arbitrating uneasily between the conflicting demands made by the turn residues and the beta-strand residues. □

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Geochemistry

Ocean islands – plums or plumes?

from Shen-su Sun

THE 'hotspot' model has played an important role in modern geological sciences ever since it was first proposed, more than 20 years ago, to explain volcanic activity in oceanic island chains, most notably the Hawaiian Islands. Morgan¹ suggested that hotspot volcanism is caused by melting of material that is welling up from deep in the mantle. Despite much research, leading to several competing models, a precise location for the sources of the zones of upwelling remains elusive. The situation presents a challenge to geochemists: can we develop tests to discriminate between the various models? In two recent papers Schilling and co-workers have addressed this question and provided firm support for the mantle plume model^{2,3}.

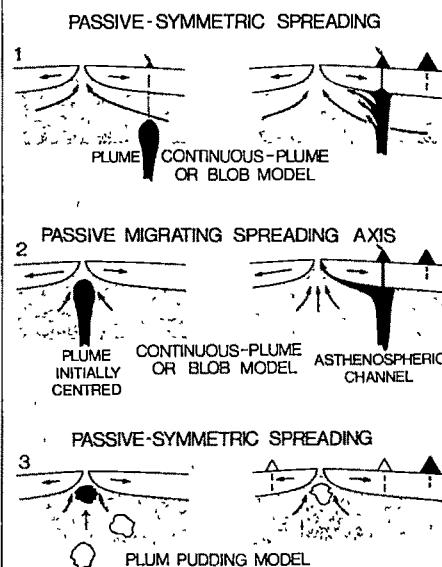
The arguments have centred on the geometric relationship between the mantle sources for hotspot volcanism and the sources for mid-ocean ridge basalts (MORB). The consensus view is that the source for MORB is located within the convecting upper mantle. The zones of upwelling judged responsible for hotspots, which Schilling *et al.* now regard as 'mantle plumes', seem to be essentially continuous and stationary in the convecting mantle with respect to the overlying lithosphere on a timescale of 100 million years^{4,5}.

Mantle plumes, according to Schilling *et al.*, are derived from 'fertile' regions capable of generating large quantities of basalt and located at depths greater than the depleted sources of MORB. Geochemical study of basalts from the Faroes-Iceland region led Schilling and Nygaard⁶ to suggest that upwelling of the plume material is cyclic on a timescale of 10–20 million years and in the form of 'blobs' instead of a continuous column. Alternative models include melting at the top of the upper mantle induced by propagating fractures caused by thermal and membrane stresses in the oceanic lithosphere⁷ or selective melting of fertile mantle domains of various sizes randomly embedded in the convecting upper mantle. The latter has been referred to as the 'raisin' or 'plum-pudding' model.

It has been known for some time that MORB dredged from 'normal' ridge segments well away from any hotspots is geochemically grossly depleted. Such depletion is characterized by low abundances of large-ion lithophile elements such as K, Rb, Th, U, Ba and Cs, together with low ratios of La / Sm and Nb / Zr, a high ratio of $^{143}\text{Nd} / ^{144}\text{Nd}$, a $^{87}\text{Sr} / ^{86}\text{Sr}$ ratio of about 0.7025 and a $^{206}\text{Pb} / ^{234}\text{Pb}$ ratio of about 18.0. In contrast, hotspot-related oceanic basalts are variably enriched in large-ion lithophile

elements and generally have higher $^{87}\text{Sr} / ^{86}\text{Sr}$, $^{206}\text{Pb} / ^{234}\text{Pb}$ and lower $^{143}\text{Nd} / ^{144}\text{Nd}$ ratios. Basalts dredged from mid-ocean ridges near hotspots show a mixing character between these two types of source. This leads Schilling *et al.* to suggest that excess plume material rising beneath the ocean island is channelled along the mid-ocean ridge axis where it interacts with the 'normal' MORB². An ideal area for testing this channelling theory is in the South Atlantic where the ocean islands of St Helena, Gough and Tristan da Cunha lie 400–800 km east of the ridge axis. The ridge axis in the South Atlantic has been drifting westward relative to the hotspots at a rate of 1–2 cm per year for 25 million years. By detecting the geochemical effect of the plume material on the mid-ocean ridge system in MORB from the rift valley at latitudes corresponding to the extension of the hotspot traces, Schilling *et al.* provide convincing support for their hypothesis.

Consideration of the geometrical relationship between MORB and hotspot sources provides further evidence for the authors' theories. As shown in the figure, models 1 and 2, which combine the mantle plume model with passive feeding of the MORB source, can account for the observations. But as Schilling *et al.* point out, the chances that the plum-pudding model (model 3 of the figure) could generate the trace-element effect observed in MORB from part of ridge axes immediately opposite the tracks of near-ridge hotspots, and not elsewhere, must be very small. Additional support for the plume model



Schematic representation of mantle flow-plate motion models. 'White' volcanoes are derived from 'white blobs'; 'black' volcanoes from 'black blobs'. Modified from ref. 2.

comes from the observation that hotspot traces cross over ridge axes such as the Ninety east Ridge and Kerguelen Island in the Indian ocean and several hotspots in the Atlantic². If the hotspots were generated by fracture propagation with their magma sources heterogeneously distributed at shallow levels in the convecting mantle, then the cross-over process should have erased all memory of thermal perturbation related to hotspots and, indeed, the hotspots themselves. Consequently, their source must be deeper than the immediate source region for MORB.

One possible snag with the proposals of Schilling *et al.* is that they require that divergent convective motion is inoperative under the ridge axis. It is not clear that this is the case. The possibility that advective overflow of the hot buoyant plume material along the connecting channel to the ridge axis could have been decoupled from the underlying convective upper mantle deserves further evaluation.

A by-product of plume material intrusion and entrainment in the depleted upper mantle is the creation of local heterogeneities — as envisaged in the plum-pudding type models. Consequently, plume and plum-pudding models need not be mutually exclusive in a dynamic mantle, which could very well satisfy geochemical and isotopic data observed in many isolated seamounts with no obvious connection to long-lived hotspot traces. That the Pb and Sr isotope compositions of basalts from Gough, Tristan da Cunha, Kerguelen Island and St Helena are unusual compared with most hotspots, offers the change of further quantitative evaluation of the plume-ridge interaction and the

plum-pudding formation process.

Finally, what about the source and cause of mantle plumes? Several models have been proposed, most of which are related to the fate of the subducted oceanic lithosphere³. If this is not smeared out by viscous flows and distributed back into the convecting upper mantle to generate ubiquitous heterogeneity, it could congregate into a distinct density layer, perhaps at the base of the upper mantle (about 650 km deep). Thermal disturbances in the deep mantle could then trigger density instabilities in this layer, inducing partial melting and plume upwelling. Such a subduction-revival cycle could take hundreds of millions of years. A spectrum ranging from pure 'chemical' to 'thermal' plumes could thus be generated according to the degree of mixing between the material derived from the subducted lithosphere and the depleted upper mantle⁴.

It is conceivable that a large plume within the slowly convecting mantle might be sufficiently robust to reach this deep layer where it could turn into a plume simply by adding an 'e' — where the 'e' stands for energy! □

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Endangered species

Salvaging single-sex populations

from Jared M. Diamond

LAST year I reported in these columns on the narrow escape from extinction of the Chatham Island Black Robin, which had declined to five males and two females (*Nature* 309, 308; 1984). The rescue of the Dusky Seaside Sparrow (*Ammospiza maritima nigrescens*) poses a greater challenge, because the three surviving individuals are all males. A programme to salvage its gene pool, directed by Charles Cook and Herbert Kale (Kale, H. W. *Bird Conservation* 1, 128; 1983 and *Florida Naturalist* 57(4), 9; 1984), illustrates both the problems encountered in such efforts and the debates over whether the efforts should be undertaken at all.

The Dusky Seaside Sparrow, a local subspecies sometimes considered to be a full species, was formerly abundant in marshes on the east coast of Florida, but in the 1960s, most of its habitat was destroyed. During the 1970s the US Fish and

Wildlife Service (USFWS) spent five million dollars to save the sparrow by buying its remaining habitat, now the St John's National Wildlife Refuge. This programme failed because of the continuing destruction of the refuge habitat by fires and drainage. The last record of a female bird was in 1976. Finally, in 1979, five of the last six surviving males were brought into captivity for their protection.

At that stage, the only chance to salvage genes of the sparrow was to breed Dusky males first with females of the related Scott's Seaside Sparrow and then with successive generations of resulting hybrid female offspring. Five such crosses would yield birds that were, progressively, 50, 75, 87.5, 93.8 and 96.9 per cent Dusky. Once a large enough flock of 96.9 per cent hybrids had been obtained, some could then be re-established in natural Dusky habitat. To this end, a Dusky male and a

Scott's female were mated experimentally in 1980, yielding four 50 per cent Dusky hybrids.

For the next two years, while the last Dusky males grew older, USFWS refused not only to fund but even to permit the hybridization programme. When the service finally granted permission in 1983, funds still had to be obtained from private sources: these were given by Walt Disney World, Florida Audubon Society, International Council for Bird Preservation and Wildlife Preservation Trust. In 1983, one 75 per cent Dusky female was produced, followed in 1984 by another 75 per cent chick (not yet sexed) plus a 87.5 per cent chick that, tragically, broke its neck. As the 1985 breeding season approaches, the fate of the Dusky gene pool depends on the three surviving males approaching 10 years of age, which is very old for small temperate-zone songbirds.

This captive breeding programme illustrates vividly two biological difficulties: that small populations are very susceptible to accidents (such as the death of the chick), and that old birds are poor breeding stock. One of the old males has been unsuccessful at wooing females, and another yielded only infertile eggs. In 1984, the 50 per cent female produced eight clutches and twenty eggs, of which only five hatched and one survived. Thus, a practical message for salvage programmes is to start when there are many youthful survivors, rather than waiting until it is too late, or nearly so.

The broader question raised by the back-crossing programme is whether it is even worth attempting. Some of the objections raised by USFWS were baseless: that hybridization would dilute the Dusky gene pool (but it was doomed otherwise); that hybrids might not be fertile (they are); and that hybrids might not resemble the Dusky Seaside Sparrow (they do). However, another objection raises a significant issue: the Service neither wants to spend money on restoring Dusky habitat for eventual releases, nor on saving hybrids. (In the words of one official, "Legally, a 98.4 per cent Dusky isn't a Dusky".) Many biologists similarly object to captive breeding programmes on single species, when money for habitat preservation that could save many species is so scarce. However, the number of species for which captive breeding or back-crossing offers the sole chance of survival is growing rapidly and the current programme on the California Condor would have been impossible without the experience gained from similar programmes with the Andean Condor and Peregrine Falcon. Thus, although the Dusky Seaside Sparrow is just a small drab bird, the lessons being learned with it now will surely have wider application. □

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Gene regulation

Controlling roles for snurps

from Phil Turner

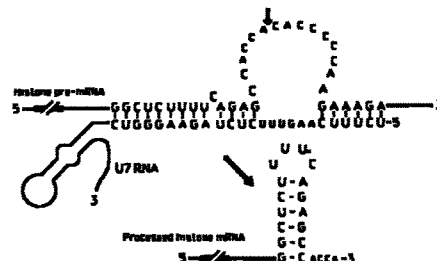
SEVERAL recent publications point to the possibility that small nuclear ribonucleoprotein particles, or snurps, may play much more important roles in the processes of gene regulation than previously thought. Over the past year or so, proof has been obtained of the involvement of snurps in the removal of introns (splicing) and the production of correct 3' ends from primary gene transcripts (pre-messenger RNA). Since there are now many examples both of alternative splicing pathways, which result in more than one mature mRNA species from a single pre-mRNA, and of the differential use of alternative poly(A) sites, snurps may not simply perform these processing steps but may be used to choose between the alternatives. If so, their action would constitute an important form of post-transcriptional control of gene expression.

Our best understanding of the biochemical reactions performed by snurps comes from the work of Max Birnstiel's group in Zurich on the involvement of the U7 snurp in the production of correct 3' ends of histone mRNA in the sea urchin. Based on the complementary DNA sequence of the sea urchin U7 RNA, Strub *et al.*¹ have suggested a model in which the 5' end of the U7 RNA hybridizes with two conserved regions at the 3' end of the histone pre-mRNA (see figure). The conserved regions in the pre-mRNA are the hairpin structure that is found immediately upstream of the 3' end of the histone mRNA in most species and a sequence (CAAGAAAGA) that occurs six nucleotides downstream of the actual 3' end of the mature mRNA. (The latter sequence seems to exist in an altered form in higher eukaryotes², possibly explaining the fortuitous requirement of the sea urchin U7 RNA for correct processing of the sea urchin histone H3 mRNA in frog oocytes³.) In this model the U7 RNA linearizes the hairpin structure by forming base pairs with 13 out of the 16 nucleotides in the hairpin. It also forms 6 base pairs with the CAAGAAAGA sequence and in doing so the region of the histone pre-mRNA between the conserved regions forms a small loop which is presumed to facilitate the endonucleolytic cleavage that produces the mature 3' end¹.

Support for this model comes both from the previous observation that the hairpin structure is absolutely required for correct 3' end production⁴ and from experiments recently reported in *The EMBO Journal*. Using a series of deletion and insertion mutants of the sea urchin histone H3 gene, Georgiev and Birnstiel⁵ have shown that the CAAGAAAGA sequence is essential for pre-mRNA processing. These experi-

ments also demonstrate that the topology of the two conserved regions is important; the insertion of only six nucleotides between them abolishes processing. In addition, for the rate of processing to be maximal, the pre-mRNA must contain at its 3' end 50–80 nucleotides with no special sequence requirement⁶ and also a 5' cap structure⁷.

This picture of the processing of histone pre-mRNA by the U7 snurp has a number of similarities to the generation of the 3' end of other gene transcripts by post-transcriptional cleavage which, however, is then followed by the addition of a poly(A) tail. In an increasing number of mRNAs, additional sequences downstream of the essential AAUAAA sequence are found to be required for 3' end formation⁸, with the poly(A) addition site falling between two required sequences. An analysis of 61 vertebrate poly(A) sites⁹ demonstrates that two classes can be described, depend-



Proposed role of U7 snurp in converting histone pre-mRNA into processed histone mRNA. Small arrow indicates the point of cleavage that generates the 3'-end of the processed histone mRNA. The two conserved sequence elements are shown in large letters. The histone coding region is the interrupted hatched line.

ing on whether the pentanucleotide CAYUG is immediately upstream or downstream of the site of poly(A) addition. Possible base-pair interactions of both these classes of poly(A) site with the U4 RNA sequence, which are similar to the suggested pairing of U7 RNA and histone pre-mRNA, have been noted⁹. Further evidence that snurps are involved in 3' processing of some polyadenylated RNAs comes from the evidence that certain antisera against snurps inhibit polyadenylation *in vitro*¹⁰.

The U4 and U6 RNAs form part of a single ribonucleoprotein particle as a result of intermolecular base pairing^{11,12} (which explains why U6 seems to react with the so-called Sm antisera despite lacking the sequence by which the Sm antigen is thought to bind). If the U4/U6 complex is involved in poly(A) processing then it must be dissociated in part for the U4 RNA to interact with the poly(A) site as proposed⁹. In this connection, the U7

RNA also seems to lack the sequence that would explain its precipitation with Sm antisera¹. Gel analysis of U7 RNA purified from a 12S snurp showed the presence of an RNA molecule about 100 nucleotides in length¹³. U5 RNA is approximately 100 nucleotides long and shares an 8-nucleotide homology with U7. The significance of these observations is not clear, but it is possible that certain snurps interact with each other during their processing activities.

Since the initial suggestion, based on sequence complementarity, that the U1 snurp might be involved in intron removal¹⁴, much supporting evidence has been obtained. Recently, Kramer *et al.*¹⁵ have shown that the ability of nuclear extracts of HeLa cells to splice pre-mRNA can be abolished by prior treatment of the extracts with human autoimmune antibodies and that the inhibition can be prevented by prior saturation of the antibodies with purified (U-type) snurps. In addition, splicing can be inhibited by removal of eight nucleotides from the 5' end of the U1 RNA of the snurp.

In a complementary series of experiments Tatei *et al.*¹⁶ have shown that U1 snurp fractions bind to synthetic single-stranded DNA 5' or 3' splice sequences or RNA transcripts derived from them, whereas U2 snurps do not. Further purification of the U1 snurp resulted in preferential loss of binding to 3' splice sites suggesting that additional components are required for U1 snurps to bind to both splice sites.

In vitro splicing of pre-mRNAs is most efficient if they possess capped 5' ends, the splicing reaction being inhibited by cap analogues as long as they are added no later than the start of the incubation¹⁷. The appearance of the spliced product, which occurs via the formation of a lariat or tailed-circular molecule (reviewed in refs 18 and 19), is only seen after a lag period of 30–45 minutes²⁰. Lag periods have also been observed for polyadenylation *in vitro*²⁰ and for histone maturation *in vivo*; the latter is cap dependent⁴. The existence of a lag period in these processing events could indicate a requirement for assembly of pre-mRNA into a suitable RNA-protein complex or a requirement for a scanning process to ensure that only appropriate 5' and 3' splice sites come together. To test whether a scanning process is involved, oligonucleotides or antisense RNA transcripts that can form hybrids with introns or exons could be introduced into processing systems; scanning might be inhibited by short double-stranded regions, whereas folding of large introns to produce lariats should not be inhibited.

The argument usually put forward against a simple scanning model is that alternative pre-mRNA splicing pathways have frequently been observed, sometimes within a single cell type²¹ and sometimes with tissue specificity²². But scanning could still be operative if, before the

commitment to splicing is taken, certain criteria at the 3' side of an intron have to be fulfilled. These criteria would include the existence of a 3' splice-site consensus sequence and, in some cases, the existence of a branch-point sequence or functional equivalent in the appropriate position²³. For some introns, the adoption of specific secondary structures could be an added criterion²⁴, and the presence of another snurp particle bound to, or aiding recognition of, the 3' splice site may also be required^{16,19}. If the various criteria were not met, splicing would not take place and the criteria would then be reassessed at the next 3' splice site. In this way certain exons could be 'jumped' or processed out. This could occur stochastically within one cell type if two alternative secondary structures were possible for that intron but only one fulfilled the criteria, or if two types of 3' snurp particles were present but only one had the necessary affinity for particular 3' splice sites to allow splicing. In such a model it would be possible for the criteria for 3' splicing to be met by a different species of pre-mRNA molecule, giving rise to the process of 'trans-splicing'. With the processing systems now available we may not have long to wait to see if this kind of reaction can occur.

If there is scanning, pre-mRNAs that undergo mutually exclusive alternative splicing, such as the troponin T pre-mRNA²², ought not to be spliced *in vitro* following pre-hybridization to an oligonucleotide complementary to an exon sequence that is usually processed out. This is readily testable. In addition, for those pre-mRNAs which exhibit alternative splicing certain parts of the precursor sequence, intron or exon, must specify in some way whether or not the 3' splice site is to be used. This could be achieved through alternative secondary structures or by differential affinities for two classes of snurps, but for tissue-specific alternative splicing it is best explained by the binding of other protein factors, which influence whether a 3' splice site will be used. In this connection it has been reported that preloading the frog oocyte nucleus with certain ribosomal proteins can alter the processing of the pre-mRNA encoding that protein (I. Bozzoni, 10th EMBO Symposium, 1984). The processing of SV40 RNA in frog oocytes also suggests that different splice sites in the same pre-mRNA have dissimilar interactions with various snurp particulars during splicing²⁵.

Similar types of regulation can be envisaged where differential use of poly(A) sites occurs. In some cases the differential use of two sites may simply reflect the relative probability of the removal of an intron that contains a poly(A) site and the subsequent use of a distal site, rather than the occurrence of processing and polyadenylation at the poly(A) site in the intron, which would remove subsequent exons from the product. Which of these two pathways was used could reflect the

endogenous levels of the different snurp activities involved. This could explain the alternative use of poly(A) sites in the immunoglobulin μ gene transcripts²⁶. Tissue-specific use of alternative poly(A) sites would require additional trans-acting factors, which would presumably recognize sequence signals in the pre-mRNA concerned, and would vary the processing pathway taken by the snurp.

If snurps do perform some of these regulatory roles (with or without the assistance of other factors), then one would expect some classes of snurps to exhibit differential regulation, as has recently been noted for U1 snurps in *Xenopus*²⁷.

In conclusion, evidence is mounting that snurp-mediated processing events are important post-transcriptional regulatory steps for some pre-mRNA types. Perhaps when the functions of U2 and U5 snurps are finally worked out we shall have a better appreciation of the subtleties of this regulation. □

Climatology

Impact of extreme events

from T. M. L. Wigley

BECAUSE the impact of climatic change is so dependent on the society that is affected, it is difficult to isolate unifying features or rules by which to assess the impact. We can be fairly sure that the early stages of any worldwide change, such as warming caused by the CO₂ greenhouse effect, will not be a problem because socio-economic factors — diversity in agriculture, international trade and planned water resources — will act as buffers to change. Impacts accrue, however, not so much from slow fluctuations in the mean, but from the tails of distribution, from extreme events. In many cases, an extreme can be defined as an event where a climatic variable exceeds some absolute threshold. This has led to the concept of climatic marginality, first studied in detail by M. L. Parry¹ and now taken further by Parry and T. R. Carter².

In analysing the effects of the severe weather of the Little Ice Age on the peoples of southeastern Scotland, Parry originally made three points; that, on the margins of agricultural viability (through cold or dryness), extreme events must occur more frequently; that the frequency of such events might change dramatically as a result of even a small change in the mean climate; and that the probability of two successive extremes is even more sensitive to small changes in the mean. The importance of successive extremes lies in their amplified cumulative impact. A single extreme may be easy to withstand but, if buffer stocks are depleted by one bad season, a second in succession could be far more devastating.

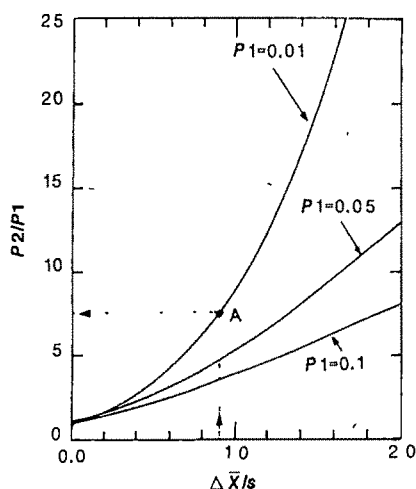
The simple probabilistic aspects of

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Parry's arguments are easy to demonstrate. Under a stable climate, an extreme event may be assumed to occur when, say, climate variable X falls below some critical threshold X_c . The probability of such an event is given by the area under the tail of the distribution where X is less than X_c . If we now shift the mean of the distribution towards X_c , this area will increase in a highly non-linear way, as shown in the figure. A change in the mean by only one standard deviation would make an extreme event that had been expected only once every twenty years become five times as frequently expected, and an event previously expected once a century would be expected every eleven years. For example, if the annual mean precipitation of about 920 mm over England and Wales fell by 100 mm (about 0.9 standard deviation) a drought that was expected one year in a hundred ($P_1 = 0.01$) would become roughly 7.5 times more frequently expected ($P_2/P_1 = 7.5$, point A in the figure). A doubling of CO₂ concentration is likely to produce changes of more than one standard deviation in both precipitation and temperature in many parts of the globe.

Changes in the probability of two successive extremes are even larger: two successive 1-in-20 year events go from being most unlikely (a return period of 400 years) to being quite probable (a return period of only 16 years). Of course, these are highly idealized calculations, but they do serve to emphasize the importance of extremes and provide a conceptual framework for estimating the impacts of future climatic change. Changes in both



Sensitivity of the frequency of extreme events to changes in the mean (based on an assumed normal distribution and constant standard deviation). Abscissa shows the change in mean (ΔX) as a multiple of standard deviation (s); ordinate shows the resulting change in the probability of extreme events with initial probabilities (P_1) of 0.1, 0.05 and 0.01.

inter-annual variability and in year-to-year persistence of climate may either amplify or dampen these influences of a changing mean. These ideas have been developed in more detail by Parry and Carter².

This focus on variability and extremes has been examined for shorter timescales by L. O. Mearns, R. W. Katz and S. H. Schneider³. Noting that some crops are sensitive to temperature extremes (for example, a few consecutive days over 95°F can significantly reduce corn yields in the United States) they have used an autoregressive model to calculate the effects of a changed mean on the

frequency of daily extremes, specifically daily maxima over 95°F. The stochastic simulation approach they use is essentially that used in stochastic hydrology, where the technique has been developed to a high degree over the past 20 years. (At least one study⁴ has considered the impact of climatic change on water resources in this way, although the results were rather inconclusive.) Echoing Parry's conclusion, Mearns *et al.* find that the relationship between changes in mean temperature and changes in the probability of daily extremes is highly non-linear, and that small changes in the mean can sometimes produce relatively large changes in the probabilities of certain events. Their results are sensitive to assumptions regarding changes in variability and changes in day-to-day autocorrelation, but the qualitative conclusions hold even if these parameters are varied.

Changes in the frequency of extreme high (or low) temperatures are of importance not only to agriculture but also, for example, to energy demand and human mortality, so this type of analysis can provide broad insights into the way that climatic change may affect society. It also opens up new avenues for multi-disciplinary research into climate impact assessment. □

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Neuropharmacology

Super-potent serotonin blockers

from Leslie L. Iversen

STARTING ON page 126 of this issue, B. P. Richardson and colleagues from Sandoz describe the discovery of a novel class of serotonin antagonists which offer new insights into the pharmacology of this biologically active indolamine¹. The new drugs are among the most potent of any type ever described: some act at concentrations as low as 10^{-14} M. The compounds, which bear a structural resemblance to both serotonin and cocaine are indoletranyls or homotropanyls esters. They specifically block the ability of serotonin to excite various peripheral nerves in the autonomic and sensory nervous systems. Furthermore, different compounds in the new series show wide variations in their potency for antagonizing the effects of serotonin on different types of peripheral nerve, suggesting that neural receptors for serotonin (termed M-receptors) comprise a variety of distinct

pharmacological sub-classes.

One important role of the M-receptors may be to mediate the painful effects of serotonin released during injury or vascular spasm, by triggering activity in the endings of pain fibres. One of the new antagonists, ICS 205-930, can block the painful effects of serotonin administered locally to human skin and may prove useful in controlling pain in migraine sufferers.

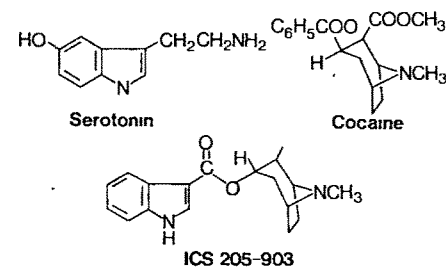
Serotonin (5-hydroxytryptamine, 5-HT) has been known for some forty years, and its pharmacological actions on various tissues have been studied intensively. Gaddum was first to show that the actions of serotonin on some smooth muscles can be blocked by the powerful hallucinogen D-lysergic acid diethylamide (D-LSD). A number of different receptor classes for serotonin has been suggested in peripheral tissues and in brain. Gaddum and Picarelli² showed that serotonin con-

100 years ago

THE COMET OF 1472. — M. Celoria of Milan has discussed the elements of the last comet observed by Toscanelli, which is the celebrated one of 1472, also observed by Regiomontanus, whose description of its path in the heavens enabled Halley to make a rough approximation to its orbit, as he states in his "Synopsis of Cometary Astronomy." The Chinese account of the comet's track contained in the supplement to the great collection of Ma T'wan Lin, of which Edouard Biot published a translation in the appendix to the "Connaissance de Temps" for 1846, enabled Langer to make a further calculation of the orbit, though the somewhat full description of the comet's course amongst the stars is unfortunately very deficient in dates.

From *Nature* 32 231, 9 July 1885

tracts the intestine by two different mechanisms: a direct action on smooth muscle, mediated by D-receptors, and an indirect action on autonomic nerves to stimulate acetylcholine release, mediated by M-receptors on neurones. More recently, radioligand binding studies have also revealed two classes of binding sites, termed 5-HT₁ and 5-HT₂. The 5-HT₁ sites are identified by high affinity binding of serotonin in brain and may mediate contractile responses in some peripheral tissues, particularly stomach. The 5-HT₂ sites, which may correspond to the D-receptors, are found in both brain and periphery; they mediate contractile responses in peripheral blood vessels and aggregation responses of blood platelets to serotonin³. These sites are potentially blocked by classical serotonin antagonists: cyproheptadine, cinanserin, methergoline, methysergide and ketanserin; in brain they are identified by using radiolabelled ketanserin as a ligand⁴. Both classes are potentially blocked by D-LSD, although it is not known whether this explains LSD's unique psychopharmacology. Neither class is sensitive to the new Sandoz analogues, which appear to be specific for the neural M-receptors.



The development of the new compounds stems in part from the discovery by J. R. Fozard⁵, that cocaine is a weak but selective antagonist of neural responses to serotonin. Fozard and his colleagues at the Merrell Dow laboratories in Strasbourg used this discovery to develop a series of selective serotonin antagonists modelled on the cocaine structure. One of these, MDL 72222, reported last year⁶,

has a selectivity similar to the antagonists now described although it is considerably less potent. The Sandoz researchers first synthesized analogues of serotonin and discovered compounds that selectively mimicked its effects on neurones or on smooth muscle. This knowledge was used to synthesize antagonists with selective action on neurones in which the serotonin side-chain nitrogen is incorporated in a tropane ring, as in cocaine (see figure).

Interest in serotonin pharmacology has been particularly strong in the past few years. New generations of antidepressant agents are emerging that act by inhibiting the re-uptake of serotonin after its release from synaptic terminals in brain, thus enhancing its synaptic actions⁷. It has also been reported recently that novel anti-anxiety drugs of the buspirone type may act by selective stimulation of serotonin receptors in brain, although the precise category of sites involved remains unclear⁸. Receptors for serotonin of the

type that are blocked by the new antagonists have not yet been identified in the central nervous system but their presence there would not be surprising.

As so often in pharmacology, the discovery of novel drugs with selective antagonist actions will undoubtedly allow rapid progress to be made in defining the occurrence and functional significance of a hitherto obscure class of monoamine receptors. □

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Surface structure

X-ray reflection from liquids

from Stuart A. Rice

IN AN important paper in *Physical Review Letters*, A. Braslau *et al.* report measurements of the surface roughness of water deduced from the scattering of X rays reflected from the surface¹. To do so, they have introduced a new way to interpret the reflectivity data. This and related advances are beginning to revolutionize the understanding of liquid surfaces.

For two decades there has been a steady stream of improvements in the scope and sensitivity of techniques with which to study the atomic and electronic structures of surfaces. Many of these techniques use electrons as probes, or detect electrons, or both. Unfortunately, because electrons interact very strongly with the atomic charge distribution, the interpretation of electron-scattering data usually requires removal of the contributions to the signal from multiple scattering processes, which cannot at present be carried out for liquid surfaces. Unlike electrons, X rays are weakly scattered by the atomic charge distribution, and two X-ray techniques have recently been introduced to study surfaces: X-ray reflectivity as a function of angle of incidence, and grazing incidence X-ray diffraction. Both can be used to study liquids as well as solids.

That X rays will be totally reflected from a condensed medium if the angle of incidence is less than some critical value was predicted and first demonstrated by A.H. Compton in the 1920s². The thrust of the work in the following decade was concerned with the determination of the refractive index of matter at X-ray frequencies, assuming the sample to be homogeneous up to a planar surface, treated as a geometrical discontinuity. It was

not until 1954 that L.G. Parratt suggested inverting the analysis and interpreting X-ray reflectivity as a function of angle of incidence via models of the inhomogeneous surface-density distribution³. This suggestion lay dormant for twenty years, until B. C. Lu and S. A. Rice⁴ adapted it to provide information about the surface structure of liquid mercury. Other applications^{5,6} are rare.

X-ray reflectivity as a function of angle of incidence is sensitive to the distribution of density perpendicular to the surface; the greater the range of incident angles for which measurements can be made, the more accurate will be the inferences drawn about the surface density profile. The dynamic range required for such experiments is large — of the order of 10^7 to 10^8 — so synchrotrons are the favoured X-ray sources. The data can be interpreted by two means. In one, a model of the surface-density distribution is assumed and the predicted X-ray reflectivity is compared with that observed. Alternatively, the data can be interpreted in terms of some model-independent parameter that is characteristic of the density profile.

It is the second means of interpretation that has been introduced by Braslau *et al.* They show that the reflectivity data they obtain can be interpreted in terms of the mean square roughness of the surface without prescribing the molecular mechanism by which the roughness is generated. The value they obtain for this measure of the surface density profile is $3.24 \pm 0.05 \text{ \AA}$. A molecular dynamics simulation of a rigid charge distribution model of water⁷ shows that the density

falls smoothly through the liquid-vapour interface with a characteristic width of $3.56 \text{ \AA}$; the deviation between these values provides one measure of the inadequacy of the model potential used in the molecular dynamics simulation.

As expected, total external reflection of X rays is accompanied by fluorescence and, very recently, this has been exploited to study the properties of solid and liquid surfaces. Smirnov⁸ has shown that measurement of the fluorescence intensity as a function of angle of incidence of the exciting X rays can be used to infer the density distribution perpendicular to the surface — in his case, for a film of germanium deposited on glass. In an independent development, Bloch *et al.*⁹ have used the same kind of measurement to infer the concentration profile of a dissolved polymer in the solution-vapour interface.

To date, the only reported application of grazing incidence X-ray diffraction to a liquid is by J. Als-Nielsen and P. S. Pershan¹⁰ who measured the interplanar spacing perpendicular to the surface of a smectic-A liquid crystal. The interpretation of the X-ray diffraction pattern in terms of the in-plane distribution of molecules in a liquid-vapour interface is more difficult than for a solid, because removal of the contribution to the X-ray scattering from atoms below the surface requires knowledge of their distribution as a function of density. Whereas the atomic distribution in a crystal substrate is substantially the same up to the outermost plane of atoms, the variation in distribution in the 2–3-atom thick, inhomogeneous liquid-vapour interface is important. Nevertheless, guided by computer simulations, it should be possible to obtain useful information about the in-plane distribution of atoms and molecules in a liquid-vapour interface.

The availability of powerful tunable X-ray sources is likely greatly to increase our information base and understanding of liquid surfaces. A few of the possibilities we can expect in the next few years are studies of the structure of films supported on liquids and of phase transitions in those films; measurements of the influence of oxidation on the structure of the liquid surface; and investigations of the spatial distribution associated with the excess surface concentration of a component of a mixture. □

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Earth sciences

Calculating continental strength from thermal history

from Marcia McNutt

SEDIMENTARY basins in continental interiors contain economically important oil, gas and coal deposits. The development of such basins has been largely controlled by the thermal history and mechanical response to sediment loading of the underlying crust and uppermost mantle (the 'continental lithosphere'). N. Kusznir and G. Karner report on page 138 the use of rock deformation data from laboratory experiments to determine directly the mechanical properties of the lithosphere from the thermal history of a basin. In as far as the extrapolation of laboratory experiments to geological loading rates remains valid, their formulation provides a mechanism for inferring the thermal parameters that govern basin development from observations of the mechanical strength of the continental lithosphere through time.

As a first approximation, the long-term mechanical behaviour of the lithosphere resembles that of an elastic plate with strength characterized by its elastic thickness T_e . Rocks above the depth T_e are strong enough to support elastically the stresses imposed by the sediment, whereas rocks below that depth deform ductilely to relax stress. The elastic thickness is primarily controlled by thermally activated creep mechanisms and hence the temperature in the lithosphere. The expectation is that a sedimentary unit deposited on hot, weak continental lithosphere will load a thin elastic plate to produce a narrow, relatively deep stratigraphic horizon. The same volume of sediment deposited on a cold, thick lithospheric plate will produce a broad, thin horizon. Therefore, stratigraphic cross-sections provide information on the mechanical strength, and by inference the thermal state, of the lithosphere during the period of basin formation.

The thermal state of the lithosphere is often summarized in terms of its thermal thickness L , the depth to which conduction as opposed to convection is the primary mechanism of heat transfer, and its thermal age t , which is the time necessary for the lithosphere to cool conductively to its present state from an initial temperature of about 1,300°C (the temperature maintained at its base). A sedimentary basin is thought to form after horizontal tractions stretch and thin the lithosphere (McKenzie, D. *Earth planet. Sci. Lett.* **40**, 25; 1978). Upwelling of hotter mantle isotherms heats the lithosphere. The subsequent cooling and subsidence of the thinned lithosphere are completely described by the initial thermal age t , the

stretching factor β , and the conductive cooling law for a slab of thickness L . Within the context of this thermal description of the continental lithosphere, Kusznir and Karner quantify the expected variations in mechanical strength of basin lithosphere.

The elastic thickness T_e of very old lithosphere (t more than one billion years) is most sensitive to the thickness of the conductive layer L . If L is 125 km — the generally accepted value for the oceanic lithosphere (Parsons, B. E. and Sclater, J. G. *J. geophys. Res.* **82**, 803; 1977) — then the greatest T_e values observed for old continental lithosphere should be about 50 km. Based on observed values of T_e in excess of 90 km for the old lithosphere beneath the Ganges and Appalachian basins, Kusznir and Karner favour a value for L closer to 225 km for continents.

The stretching factor produces observable variations in the elastic thickness by introducing perturbations to the thermal structure. For the expected range in β , extremely stretched lithosphere will seem to have an elastic thickness more than 10 km thinner than modestly stretched lithosphere for sedimentary loads deposited during the first 10 million years of basin subsidence. Decay of the thermal transient gradually eliminates any β -dependence for loads deposited 100 million years later, so that one must study the deepest layers of sedimentary basins to gain insight into the initiating mechanism.

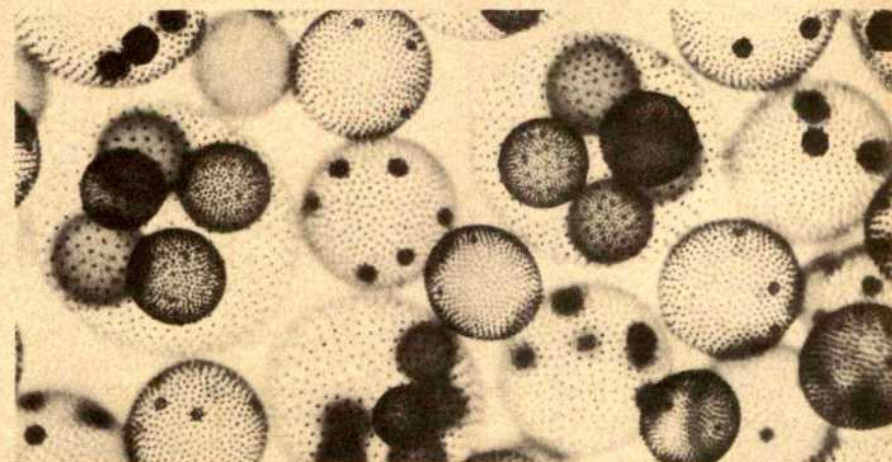
Other factors complicate the simple relationship between elastic plate thickness and thermal anomalies. For example, all rocks below the depth T_e do not relax instantaneously to relieve the stresses from sedimentary loading. Initially, the

deflection of the continental lithosphere corresponds to $T_e = L$, the full thermal thickness of the lithospheric plate. Relaxation begins at the base of the plate, rapidly at first but then asymptotically approaching T_e with time. Thermally young lithosphere ($t = 10$ million years) essentially reaches its long-term value of $T_e \approx 10$ km after only one million years because of rapid cooling of the plate. In older lithosphere ($t = 300$ million years), stresses at the base of the plate continue to relax, so that the effective elastic thickness of about 50 km decreases by a few kilometres between 1 and 10 million years. Therefore, for young loads less than 1 to 10 million years old, transient effects may cause the lithosphere to appear thicker than predicted by the steady-state theory.

The thickness of the crustal portion of the continental lithosphere is another complicating factor. The rocks in the crust, which are rich in quartz, have a lower activation energy for ductile flow than the olivine-rich rocks in the upper mantle. The lower activation energy causes ductile flow in the lower crust at fairly modest temperatures. For a given temperature structure, continental lithosphere seems weaker as the ratio of crustal to mantle portion increases. Thus two different basins both with thermal structures for 100 million-year-old lithosphere can have T_e values that differ by as much as 30 km if their crustal thicknesses differ by a factor of two.

Provided we can make allowance for the nonthermal parameters affecting the mechanical strength of the lithosphere, the elastic flexural signal preserved in sedimentary basins can be used to determine the thermal history which controls hydrocarbon formation. Kusznir and Karner's work identifies the variables and the magnitudes of the effects involved in such thermo-mechanical studies of sedimentary basins. □

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Light photomicrograph ($\times 10$) of *Volvox*. Taken by Ron Oldfield of Macquarie University in Australia, this photograph won first prize in a Polaroid Instant Photomicrography Competition.

Birth defects

Are offspring at risk from their father's exposure to toxins?

from Nigel A. Brown

CONGENITAL malformations can be induced by exposure of the mother to teratogens in early pregnancy, but can they also be caused by treatment of the father before, even long before, conception? The answer, as far as we know it, draws on observations of the effects of genetic lesions, indirectly addressed on page 144 of this issue¹, and on a consideration of potential epigenetic mechanisms.

The various stages of male germ-cell maturation can be differentially susceptible to toxic insult². It is simplest to distinguish two overall phases — pre-meiotic and post-meiotic. The pre-meiotic stem cells produce spermatogonia for the whole reproductive lifespan and they cannot be replaced if destroyed. The lifespans of post-meiotic stages are short by comparison and damaged cells will disappear within one cycle of spermatogenesis, a matter of a few weeks, whereas an abnormal stem cell may be retained for life.

It is possible for treatment of a male to induce genetic damage in germ cells which causes congenital malformation in his offspring. Male mice exposed to X rays or to the mutagen urethane produce fetuses with an increased incidence of malformations^{3,4}. Various common defects are observed, some that would be lethal after birth, others that seem just to be a reduction in fetal size. With post-meiotic exposure of germ cells, up to 4.5 per cent of fetuses are defective, whereas pre-meiotic treatment produces a lower, but still significant, incidence of defects. The malformations are probably caused by both small chromosomal aberrations and gene mutations, and are obviously genetically dominant⁴. Congenital cataracts and skeletal defects have also been observed in offspring sired many months after male mice have been exposed to X rays or ethylnitrosourea⁵⁻⁷. Breeding of these defective offspring has shown (with some technical reservations⁸) that the malformations can be inherited and so are clearly caused by dominant mutations, originally induced in spermatogonia.

Not all embryos produced by mutant spermatozoa result in malformed fetuses. Many embryos die, usually shortly after implantation, due to major aberrations in chromosome numbers or structure induced in post-meiotic stages. Structurally normal offspring can be born with phenotypic changes, ranging from minor coat colour or behavioural abnormalities to potentially lethal biochemical or functional deficits. Limited information suggests that the incidence of cancer in

mice can be increased by paternal treatment with mutagens^{3,9}.

Trasler *et al.*¹ describe birth defects produced by treating male rats with cyclophosphamide, a mutagenic drug widely used in cancer chemotherapy. They confirm that chronic low doses of cyclophosphamide produce many embryonic deaths and report a significant incidence of up to seven per cent malformed fetuses, in contrast to a previous study using a higher dose where only behavioural anomalies in offspring were observed¹⁰. A preliminary report of acute high-dose exposure has also suggested a weak increase in malformations¹¹. The current study uses small groups of treated males, so the number of abnormal fetuses is low and could be due to chance distribution of spontaneous defects, particularly as outbred rats are used. If the malformations are a genuine result of treatment, the observation is significant — many humans are exposed to equivalent doses and there is a suggestion that pre-meiotic germ cells are involved.

Epigenetic mechanisms may also be involved, as some chemicals not usually thought to be genotoxic seem also to affect offspring following paternal treatment. In rodents, paternal exposure to ethanol¹² or narcotics¹³ can lead to reductions in litter size and birthweight, neonatal survival and behavioural deficiencies, but no reproducible increase in malformations. Ethanol may have some genetic effects^{14,15} but it is far from a potent mutagen.

There is little mechanistic evidence to support paternally-induced birth defects by epigenetic events. It seems unlikely that damage to non-genetic components of sperm results in malformations, because recent micromanipulation experiments suggest that sperm cytoplasm is not essential for normal embryogenesis¹⁶. Chemicals can be transported from male to female in seminal fluid or bound to spermatozoa¹⁷ but no fetal malformations have been observed that can be attributed to treatment of gametes at fertilization or of embryos at pre-implantation stages. It seems unlikely that a chemical could retain its activity in the uterus until the embryo reaches the teratogen-sensitive stage of organogenesis.

In humans, epidemiological data suggest that certain occupations increase the risk of fathering an abnormal child¹⁸, but such data are notoriously susceptible to bias from ethnic and socio-economic factors. Two groups of men have been exposed to high levels of genotoxic agents:

survivors of the atom bomb explosions and of cancer therapy. There is no evidence of increased incidence of birth defects in children of people exposed to atomic radiation¹⁹. Men in long-term remission from cancer are less well studied but the current impression is that there is no increased risk of malformation among their children, although this is based on clinical observations with limited power to detect even a modest increase in risk. Because these men represent a unique opportunity to evaluate the possible induction of transmissible genetic damage in humans, efforts should be made to study them carefully.

There has been concern over human exposure to 'Agent Orange' although neither of its components, 2,4-D and 2,4,5-T, or the contaminant dioxin are potent mutagens. No defects have been observed in the offspring of male animals experimentally exposed to dioxin, even though it is an effective teratogen²⁰. Some studies of children of men exposed to Agent Orange have claimed that birth defects are increased but most have not, and none is large or complete enough to be definitive. Several large studies in progress, particularly of Vietnam veterans, should provide more convincing answers²¹.

It would be foolish to advise anything other than extreme caution over the exposure of humans to chemical mutagens when our understanding of the quantitative risks to future generations is so rudimentary. Many fear that the human mutational load is being increased by chemically induced recessive changes which are currently un-noticed. As far as human birth defects are concerned, the limited data are generally comforting but this is not an excuse for complacent inactivity. While we must be thankful that no known chemical induces birth defects by an epigenetic mechanism following paternal exposure, it has to be admitted that such an agent would be an intriguing tool for reproductive toxicologists. □

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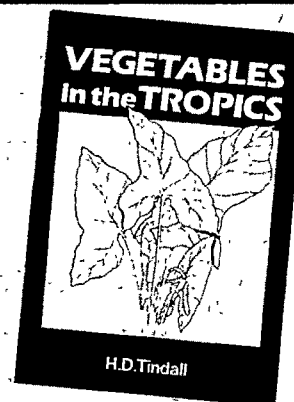
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Courses and captains

David Dickson

Lost at the Frontier: US Science and Technology Policy Adrift.

By Deborah Shapley and Rustum Roy.

ISI Press: 1985. Pp.223. Hbk \$19.95, pbk \$13.95.

THE way Western countries — and Eastern ones too, for that matter — choose to organize their science usually reflects more general attitudes about the role of the state in the economy. In Britain, for example, central planning of both spheres has always been weak, and the research system is permitted considerable autonomy, provided it observes various loosely-defined social conventions. France, in contrast, applies much stronger centralized control to its science, in line with administrative traditions that stretch back past Napoleon to Louis XIV and his adviser Colbert; while in countries such as Italy, where the state apparatus, though large and cumbersome, has less real power, the organization of science is more pragmatic and reflects initiatives taken by influential individuals.

The United States is no exception to this pattern. Largely as a result of the Congressional system, the structure of federal support for science — like that for the broader economy — results as much from efforts to balance pressures from a mosaic of frequently-conflicting lobby groups as it does from any carefully-crafted philosophy or government strategy. The evolution of scientific institutions in the United States provides a concrete testimony to the effectiveness of these pressures, whether in the form of a new research institute added to the National Institutes of Health at the prompting of a group of medical charities, or a space station eagerly promoted by sectors of the aerospace industry.

One result is that it is virtually impossible to identify a single, clear science and technology policy in the United States. Is this the most sensible way of running a research budget that is larger than the economic turnover of some European countries? Deborah Shapley, previously Washington correspondent for *Nature*, and Rustum Roy, head of the materials science laboratory at Pennsylvania State University, say no. The argument sketched out in their book *Lost at the Frontier* is that, whatever its undoubted strengths, the American research system is not contributing as much as it could be to the country's well-being, largely because it is insufficiently co-ordinated.

The book starts from the thesis that the bold plans put forward by Vannevar Bush in the years immediately following the Second World War for the renovation of American science have been betrayed by a lack of imagination and leadership. Bush

argued for increased and continuous government support for basic science — “the endless frontier”, to quote the title of his famous 1945 report — primarily as a means of achieving economic prosperity; yet this objective rapidly gave way, argue Shapley and Roy, to the philosophy that basic science was a desirable end in itself.

This “basic-research-is-best” ideology, they say, still dominates the thinking of both the scientific community and the Reagan administration. Not only has it contributed to a downgrading of research explicitly aimed at practical goals, but it has also led research workers to consider that they have a right to continued and generous support from government, regardless of whether their results are of use or value to anyone else. And this in turn has driven a wedge between the world of science and that of its technological applications which can be blamed for many of the problems currently faced by the American economy.

Even if much of what Shapley and Roy have to say about such problems has been heard many times before, their criticisms are worth repeating. It is still too easy for scientists to hide behind the argument that, since their sole motivation is intellectual, they are under no obligation either to discuss the social implications of what they are doing, or indeed to concern themselves in any way with its potential technological applications. It is also true that there are some areas of research where a greater consistency in federal support would have been more productive. One of the book's most revealing case studies concerns Roy's own field of ceramics, where wild fluctuations in government enthusiasm — and thus funding — have clearly been harmful when compared with the sustained interest that the field has received in other countries, particularly Japan.

Three central questions, however, are raised by the general thrust of Shapley and Roy's argument. Is it true that American research policy remains dominated by a “basic-research-is-best” attitude which undermines efforts to link science and technology? Would the situation be improved by a central, guiding authority? And could such an authority operate effectively within the existing institutional structure of American politics?

In each case, there is firm evidence to contradict the claims made by Shapley and Roy. On the first, for example, it is certainly true that, through a process

which started during the Carter administration and has accelerated under President Reagan, the main emphasis in government funding for science has swung back from “applied” research programmes in fields such as energy and transport towards more “basic” projects. Yet as government spokesmen frequently emphasize, this shift has taken place not for abstract, cultural reasons, but because of the twin beliefs, now widely shared in top government and industrial circles, that basic research represents a long-term investment in the technology of the future, and that the market-place — rather than a political planning committee — is the appropriate mechanism by which this investment should be realized.

Secondly, attempts to impose directions on science from the centre can be stifling rather than stimulating, however enlightened the policy-makers. Shapley and Roy describe at some length the successful efforts of Japan; but they make no mention of France, where the experience of central planning for science has been much less fruitful. Nor do they look closely at the highly decentralized (and basic-research-orientated) structure of the biomedical research community in the United States, whose vitality, when combined with the vigour and enthusiasm of the venture capital market, has been the key factor in determining America's dominant position in the global biotechnology industry.

Finally, their proposals are put forward in a political vacuum, assuming that rational discussion will lead to consensus between experts from industries, universities and government on appropriate national research priorities. In practice such priorities (ranging from synthetic fuels to the Strategic Defense Initiative) are frequently determined by non-technical arguments. The fact may drive some scientists to despair; but it needs to be seriously considered by anyone who proposes radical changes in the current pattern of decision-making. For example, in discussing the increasing restrictions placed on scientists attending conferences on topics that could have military implications, the authors describe this as merely the result of misguided political interference in the practice of science. They do not analyse in any detail the various factors that have given rise to these conflicts, such as the increasing amount of “basic” research that has a clear and direct relevance to both civilian and military technology almost simultaneously.

Shapley and Roy make several interesting suggestions about how the organization of American science could be improved. These range from various proposals for streamlining the peer review system (for example, by increasing the number of multi-annual grants and placing greater weight on prior performance), or imposing a greater concentration of government

funds on centres of research excellence, to allowing scientists to spend part of their working time doing voluntary teaching in schools (a practice recently adopted in France). Other suggestions are included in a series of responses to their case solicited from various prominent figures such as President Eisenhower's science adviser, James R. Killian, and Edwin H. Land of Polaroid camera fame. (Unfortunately most of these luminaries use the opportunity to present their own views on how science should or should not be run, rather than give detailed opinions on the ideas of the two principal authors.)

In the end, however, any arguments about the optimal strategy for science and technology which emphasize questions of internal structure, such as the relative weighting to be given to basic, "strategic" and applied research, without paying equal attention to the political logic of the external pressures which frequently determine this internal structure, inevitably appear idealistic, if not utopian. To argue, like Shapley and Roy, that American science is "lost at the frontier" and that science policy is "adrift", implies that a map and a rudder would answer the basic problems. But this raises the all-important question of how (or whether) agreement will be secured on a single map — or whose hand will be allowed on the rudder. And it can be argued that one of the most important changes in American science policy over the past decade has been the increasing influence that private industry and the military have been able to exert over precisely these processes.

There has been a long tradition of members of the scientific community declaring a strong aversion to the untidiness of the procedures by which rudimentary political issues, including the control of the direction of science and technology, tend to be resolved in society. If only, it is often argued, the organization of science — and of society too — could be made more scientific, conflicts and inefficiencies would be eliminated and the whole enterprise would immediately become more productive.

Shapley and Roy clearly share such hopes. Their optimism is to be admired, and there are sectors of the research enterprise in the United States where their solutions are certainly appropriate. But their insistence on the supremacy of scientific management risks overlooking the extent to which the cracks currently appearing in the American research system can be read as symptoms — rather than causes — of broader economic and political conflicts. It also underestimates how far these latter factors need to be taken into account if a truly rational policy for science and technology is to be achieved. □

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Truths of planetary evolution

S.R. Taylor

The Geology of the Terrestrial Planets. By Michael H. Carr, R. S. Saunders, R. G. Strom and D. E. Wilhelms. *NASA: 1984. Pp. 317. \$16 (North America), \$20 (elsewhere).*

EVERY geologist should be familiar with the landscapes and geological processes of the Moon and nearby planets, if not on account of their inherent interest then because of the insight and perspective which they shed on terrestrial geology. The authors of this volume have all made signal contributions to planetary geology, clearing away in the process much of the pre-Apollo confusion, and here provide an up-to-date and readable summary of our present understanding of the subject. Those scientists who dismiss geological evidence as merely pictorial in nature would do well to read this book and perhaps discover that hard truths about planetary evolution do emerge from the study of landscapes. Even students of cosmology should be interested to learn that the radius of Mercury shrank by a kilometre or so about 4×10^9 yr ago but, like the Moon, has remained static since that time, placing limitations on the variation of G and causing disarray among proponents of expanding Earth hypotheses.

Many questions remain to be answered. Are the smooth plains of Mercury really analogous to lunar maria or are they ejecta blankets? The composition of Mercury is clearly distinct from that of the Moon, and the recent failure to confirm, by reflectance spectra, the presence of Fe^{2+} on the surface must arouse doubts about their volcanic nature. A further problem is whether heavily cratered surfaces, on Mercury and in the lunar highlands especially, are saturated with craters, or constitute a production population. In the latter case, we can read the history of the surface since accretion. The former possibility, that we see only the record of the closing stages of the great meteoritic bombardment, implies that the observed lunar highland surface is no older than 4.1 or 4.2×10^9 yr. This seems more likely to me. Equally contentious is the existence of the giant Procellarum basin, 3,200 km in diameter, which may underlie much of the visible face of the Moon. Wilhelms's overview of lunar multi-ring basin stratigraphy removes the basis for the once popular "lunar cataclysm" hypothesis. The nature of the Tharsis bulge on Mars, whether a plateau uplifted by internal forces, or constructed by volcanic flows, remains open, but evidence for the former presence of flowing water on Mars seems unequivocal. The surface of Venus is perhaps analogous to

earliest Archaean times on the Earth. Evidence of granitic or other siliceous rocks, so typical of the continental crust of the Earth, appears to be minimal for Venus and the other planets; such rock types, familiar even to city dwellers as facings of buildings, may thus be uniquely terrestrial, a consequence of the abundance of liquid water.

The geological study of the terrestrial planets continues to flourish. More recent discoveries of many multi-ring basins on Mercury and the possibility that the lunar megaregolith is 25 km deep do not appear in the book, but it is nonetheless commendably up to date. Overall editorial control appears to have been satisfactorily light, so that the expertise and style of the individual authors (Strom on Mercury, Saunders and Carr on Venus, Carr on the Earth and Mars, and Wilhelms on the Moon) show through clearly.

Minor failings include an inadequate index of three pages (partly offset by a four-page table of contents). References are given at the end of the book, by chapter, but a combined reference list would have been preferable. Don Wilhelms will be especially chagrined to find that his beautiful plates (C and D on pp. 202–205) of the palaeogeography of the Moon have been transposed. And the maps, 18 pages of them at the end of the book, are really too small; NASA, unconstrained by the limitations of commercial publishers, would have been better advised to have included their useful larger series of planetary maps in a pocket.

This is a valuable contribution to the literature dealing with the terrestrial planets. It provides an excellent basis for university courses in the geology of the terrestrial planets as well as solid reading for geologists involved in the subject. □

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Chemical reference in English

Two of the oldest and best known reference works for chemists, *Beilstein Handbook of Organic Chemistry* and *Ullmann's Encyclopedia of Industrial Chemistry*, published in German for 104 years and 71 years, respectively, are now appearing in English.

Beilstein consists of a main series and five supplementary series. Publication in English has started with Vols 17–27 (heterocycles) of the fifth supplementary series (EV) of the fourth edition, covering the literature from 1960 to 1979. Executive editor of the work as a whole is R. Luckenbach of the Beilstein Institut, publisher is Springer-Verlag.

Ullmann's in English is to be a 36-volume series — 28 "A" volumes (alphabetically arranged articles) and 8 "B" volumes (basic knowledge), to appear at the rate of three or four a year — and commenced with Vol. A1 of the fifth edition, *Abrasives to Aluminum Oxide*. Executive editor is W. Gerhartz and publisher is VCH.

It came from space

Peter Kemp

Comet Halley: A Novel. By Fred Hoyle. Michael Joseph: 1985. Pp. 410. £9.95. To be published in the United States later this year by St Martin's Press.

WHAT'S most astronomical about Fred Hoyle's new novel, *Comet Halley*, is the number of different subjects packed into its pages. The book sets out to be a love-story and a political satire, a thriller, a spy mystery, an exercise in science fiction, a pacifist tract, a comedy of Cambridge manners and an essay in evolutionary theory. Like the heavenly body at the heart of its narrative, it whizzes along — sometimes garish, sometimes gaseous — throwing off material in all directions.

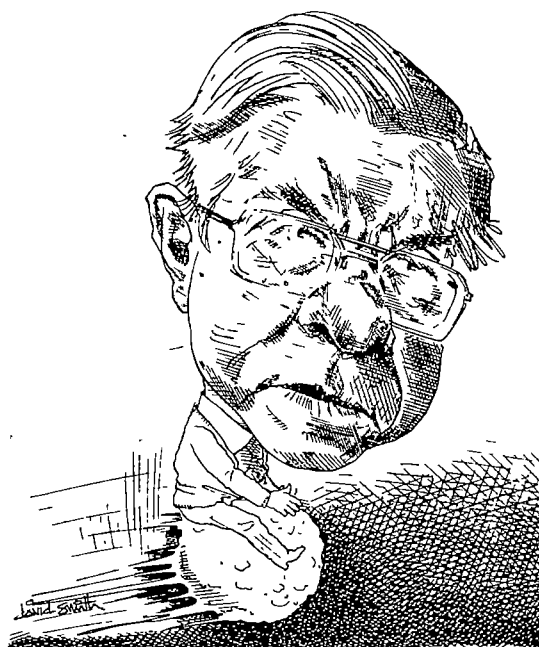
The story opens with scientists in Cambridge, England, amazingly receiving radio messages beamed from Comet Halley. As excitement about this mounts, so does the local death-rate. Researchers expire in unusual circumstances; there are murders, lethal booby-traps, a deadly bombing; several characters are found mysteriously charred to death. Gradually sorting out what's behind all this is the improbably-named Isaac Newton: no relation to the seventeenth-century luminary but like him in opening up a new dimension in thinking. For, along with Frances Margaret, a brainy beauty who becomes his lover, he reaches the conclusion that Comet Halley harbours life — and life of a civilized and civilizing disposition. Comprising "a biologically generated nuclear reactor", it seems, the comet is directing itself to the prevention of nuclear war on Earth.

Soon, in his efforts to assist the comet in its pacific purposes, Newton is striding down the corridors of power (echoes of C. P. Snow keep reverberating through this ambitious sprawl of a novel), trying to convince politicians that humanity is on the brink of a momentous breakthrough. If only nations would realize the folly of pouring money into military budgets and divert resources into the building of telescopes instead, it's argued, then they will be able to receive and re-direct messages from Halley and a host of other would-be communicative comets throughout the Galaxy. The Earth, Newton points out, could become the hub of "a sort of cosmic telephone exchange", "an extra-terrestrial communications system". Since each comet is "a giant brain by our standards", the linking of them all into one vast network of brainwaves must result in hitherto undreamed-of intellectual advances.

Factors standing in the way of this — scepticism, militaristic bigotry, the stockpiling of nuclear weapons — are robustly guyed and decried in this book. Likewise, those who attempt to deflect Comet

Halley's benign aims find themselves having a hard time. When not actually reduced to blackened stalagmites by long-distance emissions from the peace-loving projectile, they succumb to curious maladies: in Moscow, the Politburo go down with "the mad itch", an extra-terrestrial ailment that plagues people into frenzies of nude scratching; the population of Washington is afflicted by a strange derangement which brings on an irresistible urge to hibernate. Finally, ramming its aims home with some emphasis, the comet bombards the Arctic with a guided missile, pulverizing the ice-cap and hastening a change in the climate of opinion on Earth by drastically altering the planet's weather.

As if this weren't striking enough, Hoyle throws in further excitements for good measure: there's a particularly grisly sub-plot involving "a flying torture chamber" staffed by a quartet of odd-shaped psychopaths: a sadist with a Neanderthal skull and larger teeth than is customary, an ape-like giant, a vicious dwarf and a "ghastly waxen-faced creature" with shoulder-length white hair who wields a syringe filled with some unbearably agonizing concoction. Injecting one dramatic incident after another into his plot, Hoyle is generous to



a fault with dramatic incident — but always for didactic purposes. In as eye-catching a way as possible, *Comet Halley* is designed to trail its author's views on the emergence of life — and the likely extinction of it on Earth unless there's a change in thinking. □

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Grains of evidence

Peter D. Moore

The Palynology of Archaeological Sites. By Geoffrey W. Dimbleby. Academic: 1985. Pp. 176. \$45, £34.50.

POLLEN analysis has proved a valuable asset in the investigation of archaeological sites, though the problems involved in its application have not always been adequately appreciated. Only rarely are such sites conveniently located close to deep lake or peat sediments which permit extensive environmental reconstruction; more often the archaeologist has to make do with a buried soil, an infilled ditch or a compacted cave deposit. And such unconventional sources require specialized techniques of sampling and interpretation.

Geoffrey Dimbleby has been the pioneer in this field and has now brought together the collated wisdom of his years of research. It is a book with a strong practical emphasis, reflecting the fact that Dimbleby has been concerned not only with the laboratory analysis of samples but also with the collection of materials in the field. Such field experience shows through in the discussion of the various sources of polleniferous material and in the helpful warnings given about the limitations

that are imposed by different site factors.

Very naturally, much of the book is given over to soil pollen, Dimbleby's principal interest. This subject, which has been neglected and undervalued by most palynologists, has long required the kind of detailed and frank scrutiny which Dimbleby has here provided. He examines the many processes, such as the downwash and the faunal mixing of pollen, which prevent the simple interpretation of soil pollen profiles as stratified sequences.

As well as dealing with interpretive problems, the book contains many of Dimbleby's soil pollen diagrams which illustrate the kind of information to be gleaned from soil pollen profiles. Perhaps this aspect should have had less prominence, however, since these diagrams are readily available elsewhere and undoubtedly contributed to the book's high cost.

The author's cautious attitude to the application of pollen analysis and interpretation of the results may dismay some archaeologists who expect rather too much from the technique. But the book should be welcomed as a timely damper on those exuberant spirits whose enthusiasm outpaces their data. □

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Going through the changes

Mark Johnson

On the Nature of Human Plasticity. By Richard M. Lerner. Cambridge University Press: 1984. Pp.208. £17.50, \$29.95.

"PLASTICITY" is one of those labels that has taken on a variety of different, but overlapping, meanings. For example, the neurophysiologist may use the term to refer to the capacity of the nervous system to regenerate or reorganize after injury or environmental change, while some psychologists choose to reserve the term for the possible range of variation that can occur in individual development. Because of this confusion of meanings, it is essential that anyone setting out to integrate information on "plasticity" from various disciplines (in this case genetics, neurobiology, evolution, individual and group psychology) should adopt a clear and precise definition. In his book, however, Richard Lerner appears to be content to allow his interpretation of the word to emerge from the interaction between the reader and the 174 pages of text. This is both a strength and a weakness. While it enables the author to apply the term to everything from molecular genetics to social relationships, the price to be paid is the rather doubtful usefulness of his conception within some levels of explanation.

Two main theses with regard to human development are put forward in the book. First, Lerner suggests that "plasticity" is not confined to the early years but exists throughout the entire life-span. The "life-span" developmental view is a recent school of thought in psychology which emphasizes the capacity for change in the adult. This emphasis on the adult allows its proponents, including Lerner, to use "plasticity" to refer to any changes in structure or function in the organism. However, when this conception of the term is confused with "plasticity" as a specific response to the environment, which it is in the book, the reader is left with the implication that all developmental change is environmentally specified. And this while claiming to have moved beyond the old nature-nurture debate.

The second main thesis is that variables and processes at different levels (biological, individual psychological, dyadic, social and so on) may contribute to human functioning at any one time. These levels do not work independently, in parallel, but are reciprocally influential. Each level is seen to be "embedded" within the others. Lerner formalizes this idea in a framework for describing the development of individuals within their multi-levelled environment. However, as he himself admits, this framework merely constitutes a specification of the domain

within which future theory and research about individual development should be generated. Its usefulness can only be assessed by the success or otherwise of the models it gives rise to. Even if successful at the psychological level, it is not clear whether such a framework would be appropriate at, for example, the neurobiological level. Equivalence of terminology does not imply equivalence of mechanism.

Lerner sees the ideas presented in the book as being of consequence for the clinician. Since each "level" is engaged in dynamic interaction with the others in which it is embedded, it follows that intervention by the clinician at one level will have effects at other levels. For example, changing an individual's diet may have an influence on neurotransmitters which may then have effects on behaviour, and

thence on dyadic and other social relationships. The effects at other levels may be beneficial or detrimental, but in order to predict them some understanding of the mechanisms of interaction between levels is required. Lerner offers us only description, and description is not adequate for prediction.

In his foreword, Paul Baltes states that despite his approval of the book he is convinced that the author will be criticized for his commitment to interdisciplinary breadth. On the contrary, such a commitment is to be applauded. However, the synthesis we are offered in this book is, like all mirages, attractive at a distance but elusive close up. □

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Nonsenses of today

Joseph Silk

Perfect Symmetry: The Search for the Beginning of Time. By Heinz R. Pagels. Simon & Schuster. 1985. Pp. 329. \$18.95. To be published in Britain later this year by Michael Joseph.

PARTICLE physics and cosmology have developed a symbiotic relationship. While it would take a particle accelerator stretching from here to Alpha Centauri to smash particles together at energies approaching the grand unification scale (and we may safely consider this to be outside the future budgets of CERN or Fermilab), such energies were reached routinely in the very early Universe. Hence the attention being paid by particle theorists to constraints from cosmology. Already, many exotic theories have been eliminated, and others, phoenix-like, have arisen. Does the dark matter that pervades the visible Universe consist of massive chunks of quark matter at nuclear density? Or does it consist of shadow matter, a whole universe of stars and galaxies, invisible to us apart from their gravitational interaction?

This book by Heinz Pagels, a particle physicist, provides a lucid introduction to the interplay between cosmology and particle physics. He describes the motivation and basis for grand unification theories, and their role in cosmology, and includes a chapter on the inflation of the Universe, one of the most notable results of the synthesis between particle physics and cosmology. Inflationary cosmology provides a scientific basis for understanding why the Universe is so large, and why it is so isotropic (that is, similar in all directions). It explains why the Universe is almost, but not quite, uniform: from the primordial fluctuations in density, the galaxies eventually grew.

Although the hottest topics from last year's conference season, such as quark nuggets or shadow matter, are inevitably absent, there is a sufficiently solid introduction to the new physics that the reader will have little difficulty in making sense of the latest developments that appear in the semi-popular press. But to give him full credit, Pagels does not hesitate to tackle some vertiginous issues. What happened before the big bang? What was the origin of everything? Our Universe may well have begun as a quantum fluctuation: before, there was nothing, not even time or space, but absolutely nothing.

Much of the text is devoted to an account of the astronomer's Universe. The latest discoveries from space astronomy are described, ranging from the infrared-emitting embryo stars being hatched deep within dense interstellar gas clouds to the tenuous hot plasma glowing in X-rays throughout megaparsecs of intergalactic space in the great galaxy clusters. All of this, and a capsule description of the evolution of the big bang, from the beginning of the knowable Universe, some 10^{-43} s after the initial singularity, until about 1×10^{10} yr later.

The book is written for the intelligent layman, and thus non-technical and not unduly laden with jargon. It is, however, replete with quotations that help communicate the excitement at the frontier of science. My favourite is Eddington's reaction to the discovery of the first white dwarf star, Sirius B, supposedly made of material thousands of times denser than any known matter: "Shut up. Don't talk nonsense". Eddington's comment has been repeated many times since in reaction to novel observations or experimental data: seventy years later, it remains to be seen whether we have truly learned our lesson. □

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Lorentz resonances and the structure of the jovian ring

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Charged dust orbiting through spatially periodic planetary magnetic fields will experience time-variable electromagnetic forces. When the forcing frequencies are nearly commensurate with the particle's orbital frequency, the particle undergoes large out-of-plane and radial excursions. Specific 'Lorentz' resonances, corresponding to particular spatial periodicities in the magnetic field, occur on either side of synchronous orbit. We describe here Lorentz resonance locations and strengths for the jovian and saturnian rings. The boundaries of the halo of the jovian ring, and perhaps other ring structure, are near resonances.

ORBITAL resonances, those positions at which periodicities in perturbations are commensurate with the orbital period of a test particle, have long been suspected to govern much of the structure of the Solar System. For example, gaps and concentrations are found in the asteroid belt at positions where the orbital period of a particle would be close to a simple fraction of Jupiter's orbital period^{1,2}. Similarly, the Voyager spacecraft discovered some 50 cases of ring edges, isolated narrow ringlets, gaps or wave trains in Saturn's rings located at resonances with nearby satellites^{3,4}. On the other hand, it is unlikely that the uranian rings reside at three-body resonances (as once suspected⁵) because the putative resonances are too weak^{6,7}. As well as the above resonances, which are produced by the periodic pulls of orbiting perturbers, 'lumpiness' in a planetary gravitational field could affect particles having orbital periods that are fractions of the planet's spin period⁸ (compare with ref. 9); but recent refinements in ring dimensions show no unusual features at such locations³.

Only in the case of Jupiter's ring¹⁰ have resonances not been invoked extensively (see ref. 11), perhaps because satellite perturbations are weak since neighbouring moons are either distant or small, and because collective gravitational effects are thought to be non-existent in such a tenuous structure¹⁰. We show here that several features of the jovian rings are, however, near 'Lorentz' resonances and we outline how this connection might be enforced^{10,12}. We also point out that the ring's outer edge is close to a feeble satellite resonance; if this association is confirmed by the Galileo spacecraft, the workings of resonances may have to be reappraised.

Lorentz resonances

Electromagnetic forces have been shown to be significant for jovian ring particles, especially as the possible cause for out-of-plane motion^{10,12-15}. Accelerations $[(q/mc)\mathbf{v} \times \mathbf{B}]$ resulting from Lorentz forces are fairly strong perturbations because: (1) jovian particles have large velocities $\mathbf{v}$ across an intense magnetic field $\mathbf{B}$; and (2) their electric charge-to-mass ratios q/m are generally larger than for members of other ring systems because most visible jovian grains are tiny (of the order of a micrometre^{10,12}) and adopt an electric potential V determined by the surrounding magnetospheric plasma and estimated at -10 V (refs 10, 16, 17). The jovian magnetic field is far from an aligned dipole^{18,19}; in a multipole representation, the quadrupole and octupole terms contain 30% of the strength¹⁸. Accordingly, the Lorentz force on a jovian ring particle varies substantially even for a particle of fixed charge travelling along an equatorial circular orbit. Specifically, a particle in a pure dipole field would experience varying radial forces if the dipole were tilted or were offset equatorially; a constant latitudinal force would be produced by an axially offset field and a variable one by a tilted field.

A particle orbiting a planet with a spatially periodic magnetic field will experience a temporally-variable electromagnetic force

as it passes through the various frequency components of the field. Lorentz resonances are said to occur at those positions where orbital periodicities match a periodic component of the Lorentz force, for then the perturbing force will be in phase with the particle's natural oscillatory motion. This elementary viewpoint has been supported by a perturbation solution¹⁵, where the equations of motion are found to be those of forced, linear harmonic oscillators. To locate Lorentz resonances, we assume that in the neighbourhood of the ring the jovian magnetic field $\mathbf{B}$ can be derived from a potential function ψ (refs 18, 19) due either to current sources in the planet or at great distances from it. ψ can be represented by an infinite series of spherical harmonic functions, such that for internal sources

$$\mathbf{B} = -R\nabla \sum_{l=1}^{\infty} (R/r)^{l+1} \sum_{k=0}^l P_l^k(\cos \theta) [g_l^k \cos k\phi + h_l^k \sin k\phi] \quad (1)$$

where R is the planet's equatorial radius, (r, θ, ϕ) are the usual spherical polar coordinates measured relative to the rotating planet, $P_l^k(\cos \theta)$ are the Schmidt-normalized associated Legendre functions, and g_l^k, h_l^k are the observationally-determined Schmidt coefficients defining the field.

As each component of the field is a trigonometrical function of the longitude ϕ , an equatorial particle moving with a mean orbital angular velocity n would experience the k th spatial component of the field to vary with a frequency $k(n - \Omega)$, where Ω is the spin rate of the magnetic field (planet). Lorentz resonances are present at those radial positions where this frequency matches local epicyclic frequencies, defined as the rates κ and μ at which a test particle displaced by an arbitrary small amount will oscillate about the reference circular orbit in the horizontal or vertical directions²⁰. Ignoring planetary oblateness for the moment, the horizontal (κ) and vertical (μ) epicyclic rates equal n . Hence the resonance condition is approximately

$$k(n_k - \Omega) = n_k \quad (2)$$

where n_k is the mean motion of a particle at the k th resonance; a positive k is appropriate if $n_k > \Omega$ (that is, resonances interior to synchronous orbit defined as the radius R_{syn} where $n = \Omega$), whereas a negative k corresponds to exterior resonances. Morfill and Grün²¹ have found independently a similar resonance in connection with the motion of charged dust across the sector structure of the interplanetary magnetic field.

Figure 1 illustrates the operation of a typical Lorentz resonance pair. The radial portion of the $k=2$ magnetic field component is outward in two quadrants and inward for the remaining two, if we define zero longitude such that $g_2^1 \equiv 0$ in equation (1); although the strength of the $k=2$ component varies sinusoidally across a quadrant, that is unimportant for this heuristic description. Negatively charged particles at the interior and exterior resonant orbits each experience out-of-plane Lorentz forces. The force on the interior particle at the location

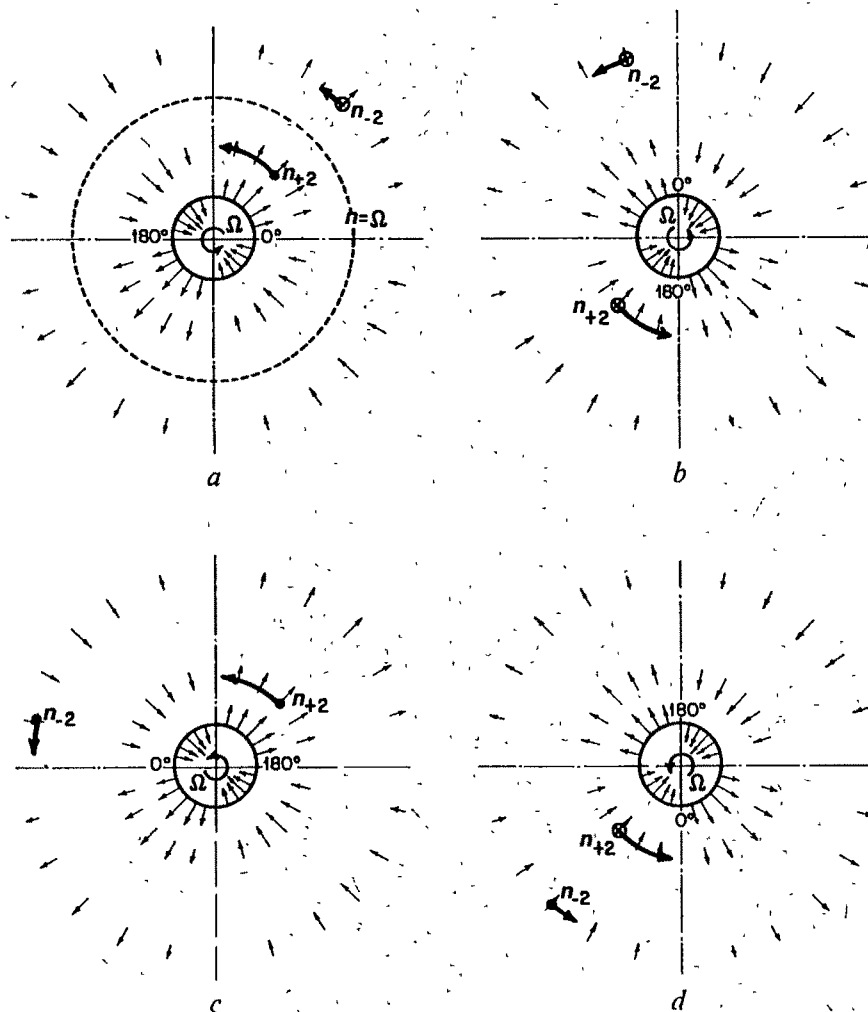


Fig. 1 Two equatorial particles—one at the interior $k=+2$ Lorentz resonance and another at the exterior $k=-2$ resonance—in four views from the North Pole. The outward and inward arrows represent the radial part of the planet's magnetic field that has $k=2$ periodicity; the planet, drawn as a solid circle, rotates by $\pi/2$ (relative to inertial space) in each successive panel. For each of the two orbits the Lorentz forces ($\bullet$, out of the plane of the paper and $\oplus$, into the plane of the paper) change sign every π radians of inertial space; these forces thereby induce north-south motions, that is, they produce inclined orbits. Large radial excursions of the orbit will be similarly generated because the latitudinal field also contains a $k=2$ periodicity. The synchronous orbit ($\Omega=n$) is shown dotted in *a*.

shown in Fig. 1*a* is out of the plane of the paper (as v , the velocity relative to the field, is positive) but the force on the exterior particle at the same longitude is into the plane of the paper (as relative v is then negative).

In Fig. 1*b*, the magnetic field has rotated by $\pi/2$, and the interior particle (having orbited through π relative to inertial space) now feels a southward force. The other particle, having travelled only $\pi/3$ radians, still remains in the first quadrant with its outward-pointing field, and so its force remains southward, although smaller than initially.

After another quarter rotation of the planet (Fig. 1*c*), the inner particle is back to its starting point (but now above the opposite face of the planet). The force on the particle at the $k=-2$ resonance has finally reversed, so both are subject to southward forces.

In Fig. 1*d*, the two particles are aligned once again, exactly π from their configuration in Fig. 1*a*, and both experience forces with opposite sign from one another and from those in Fig. 1*a*. The cycle now repeats itself. During its 3π longitudinal excursion, the interior particle has been pushed up and down twice, whereas the particle at the exterior resonance has only travelled π radians and gone through half a force cycle. Each has been pushed up on one side of its orbit and down on the opposite side exactly in phase with the orbital motion.

In terms of equation (2), the interior resonance of Fig. 1 is found at that radius where the mean motion satisfied $2(n_{+2}-\Omega)=n_{+2}$, or $n_{+2}=2\Omega$, whereas the exterior resonance is defined by $-2(n_{-2}-\Omega)=n_{-2}$, or $n_{-2}=(2/3)\Omega$; as already shown in Fig. 1, $n_{+2}/n_{-2}=3$. Clearly, the $k=+1$ (interior) resonances can never be achieved because the perturbation frequency observed by the particle $(n-\Omega)$ is everywhere $<n$. Furthermore, resonances become more and more closely spaced (but generally also

weaker as we will show) when the synchronous orbit position is approached from either side.

Using Kepler's third law to express n in terms of r , the k th resonance is approximately located at

$$r_k = \left[\frac{k-1}{k} \right]^{2/3} R_{\text{syn}} \quad (3)$$

The interior k th resonance is found by substituting positive integers while the exterior k th resonance is located with negative integers.

R_{syn} can be calculated to be at $2.2444R_J$ for Jupiter, while for Saturn $R_{\text{syn}}=1.8651R_S$, if the effects of the higher-order gravitational moments J_2 and J_4 are included.

To locate the resonances more precisely, the expressions for the epicyclic frequencies κ and μ should include higher-order gravitational moments, which cause $\kappa < n < \mu$ and, therefore, mean that the vertical and horizontal resonance positions split. Substituting classical expressions²² for n , μ and κ , accurate to J_2 , into equation (2) with the right-hand side replaced by μ , the k th vertical Lorentz resonances are at

$$r_{kv} = [(1-k^{-1}) + (3/4)(1-3k^{-1})J_2(R/r_k)^2]^{2/3} \times R_{\text{syn}}/[1 + (3/4)J_2(R/R_{\text{syn}})^2]^{2/3} \quad (4)$$

Horizontal Lorentz resonances are found by replacing n on the right-hand side of equation (2) by κ to yield

$$r_{kh} = [(1-k^{-1}) + (3/4)(1+k^{-1})J_2(R/r_k)^2]^{2/3} \times R_{\text{syn}}/[1 + (3/4)J_2(R/R_{\text{syn}})^2]^{2/3} \quad (5)$$

(Note that the signs of the second and fourth terms for κ are wrong in ref. 22.) Expressions (4) and (5) are equally valid for

interior and exterior resonances as long as k is taken to have a positive and negative sign, respectively. As $J_2 \ll 0(10^{-2})$ for all planets, these expressions can be solved rapidly by successive approximation. More exact expressions for n , κ and μ involve J_4 and J_2 (ref. 2), which introduce corrections of $0(10^{-4}-10^{-5})$ to equations (4) and (5). These terms have not been included because comparable or larger corrections will arise by direct electromagnetic effects: n itself will depend on the particle's charge-to-mass ratio because centrifugal balance should include the constant radial component of the Lorentz force, which generally will shift resonances by small amounts that depend on k .

To a lesser extent, the exact positions of the resonances are uncertain because the various harmonics of the magnetic field might rotate at slightly different rates (D. J. Stevenson, personal communication); for example, the non-dipole part of the Earth's field drifts westward relative to the surface at $\Delta\Omega/\Omega \sim 10^{-5}$, and, accordingly, resonant positions would be translocated by a comparable fraction.

We estimate the relative strength of the k th Lorentz resonance from the latitudinal force that has k periodicity. This is proportional to $v_{\phi k} B_{\phi k}$, where $v_{\phi k} \equiv r_k(n_k - \Omega)$ is the longitudinal speed of the particle across the part of the radial magnetic field B_r that has k periodicity. We estimate $B_{\phi k}$ by the term in equation (1) that has k periodicity and the lowest power of R/r_k . The coefficient of this term is $(|k|+1)(R/r)^{|k|+2} P_k^k(0) C_k$, where C_k is defined as $[(g_k^2) + (h_k^2)]^{1/2}$ and serves as a measure of the relative importance of the k th component of the field. Hence the strength S_k of the k th resonance is proportional to

$$S_k \sim \frac{(|k|+1)}{(1-k)} \Omega R (R/r)^{|k|+1} P_k^k(0) C_k \quad (6)$$

This expression pertains to latitudinal forces; the radial forces are comparable.

The resonances that we have just described are appropriate for the linearized problem. A more complete treatment would incorporate nonlinear terms, including a coupling between the radial and latitudinal motions. Based on the classical theory of coupled, forced nonlinear oscillators, we anticipate that the solution to the full nonlinear problem will contain features not present in the strictly linearized equations. The locations of the higher-order (nonlinear) horizontal Lorentz resonances can be found by generalizing equation (2):

$$k(n_k - \Omega) = K n_k \quad (7)$$

where K is a small integer; for vertical Lorentz resonances, the right-hand side of equation (7) would also be $K n_k$ with the appropriate higher-order corrections included in n_k . Because of non-linearity, super- and sub-harmonic resonance motions might be achieved at such positions. New locations are introduced through equation (7) and the previous positions of equation (4) can be shifted slightly because the 4:2 resonance does not lie on top of the 2:1 resonance since higher-order corrections enter them differently.

Resonance locations and strengths

The locations of the low-order Lorentz resonances for Jupiter and Saturn according to equations (4) and (5) are given in Table 1, where we have taken $J_2 = 0.01473$, $J_4 = -0.00059$ and $R_J = 71,398$ km for Jupiter²³, and $J_2 = 0.01630$, $J_4 = -0.00092$ and $R_S = 60,330$ km for Saturn²⁴; J_4 is used only in computing R_{syn} but not in equations (4) and (5). In agreement with our earlier statement, we see that a vertical $k = +1$ resonance is not permitted; the horizontal $k = +1$ resonance has a solution that will lie within the surface of the planet unless the planet is unreasonably oblate and dense (J. J. Lissauer, personal communication).

Relative strengths for the various k resonances in the jovian and saturnian ring systems are also listed in Table 1. To represent the planetary magnetic fields we have used the Pioneer 10 (3I, 2E) model¹⁹ for Jupiter since that spacecraft traversed a nearly equatorial orbit in the region of interest, and the JGR80 model for Saturn^{25,26} as it is the only representation involving

Table 1 Locations and relative strengths of Lorentz resonances

Resonance order k	Jupiter ($1 R_J = 71,398$ km)		Saturn ($1 R_S = 60,330$ km)	
	Location r_k/R_J	Relative strength*	Location r_k/R_S	Relative strength*
interior				
+1	—	—	—	—
+2	V 1.4065 H 1.4276	4,004	1.1653 1.1930	155
+3	V 1.7102 H 1.7190	629	1.4200 1.4315	22
+4	V 1.8513 H 1.8565	~200†	1.5378 1.5447	~10†
R_{syn}	↑ 2.2444		↑ 1.8651	
exterior				
-4	↓ V 2.6046 H 2.6023	~20†	↓ 2.1648 2.1615	~1†
-3	V 2.7188 H 2.7163	49	2.2596 2.2560	2
-2	V 2.9408 H 2.9375	145	2.4438 2.4394	6
-1	V 3.5618 H 3.5575	574	2.9592 2.9537	3

* Based on equation (6), using r_k as given here and magnetic field coefficients estimated from refs 19, 26. The tabulated values are 10^4 times those computed from equation (6); so as to have non-dimensional results that can be compared, we have not included ΩR in equation (6) for Jupiter and have divided the Saturn result by ΩR of Jupiter.

† The appropriate magnetic field coefficients are unknown and are arbitrarily selected to be similar to the planet's octupole terms

$k = 3$ sectorial harmonics. The particular values for the spherical harmonic coefficients are not especially accurate, especially for the higher-order terms; in fact, the fields probably change over long times as does the Earth's. Nevertheless, we emphasize that although resonance *strengths* will vary, depending on the precise representation selected for the magnetic field (see refs. 18, 19), the resonance *locations* rely merely on the spatial periodicity of the field, which depends on the well-demonstrated adequacy of the spherical harmonic model.

Compared with Jupiter's gravitational attraction, the total Lorentz force magnitude at the k th resonance is $0.008 |1/(1-k)| (V/10 \text{ volt}) (\mu\text{m/s})^2 (2g \text{ cm}^{-3}/\rho)$, where s is the particle's radius and ρ is its mass density; this assumes that field emission does not limit the electric potential of the particle, for otherwise s would appear to the power -1 (refs 10, 16). The component driving the $k = +2$ Lorentz resonance is about one-tenth the total Lorentz force. At the other resonances further from the planet, electromagnetic forces are somewhat less important because the higher-order components of the magnetic field decrease more rapidly than gravity does and because the speed of the particle across the field is generally lower.

As with any simple resonance, a region surrounding the precise resonance location shows amplified motions because the frequency of the force nearly matches the natural frequency of the system's response. Figure 2b depicts this by plotting the response of the system to typical forcing terms across the region of Jupiter's ring; this response is $\sim S_k [n_k^2 - k^2(n_k - \Omega)^2]^{-1}$ for each of the components. Note that the low-order resonances are quite broad (for example, the resonant term for the $k = +2$ case is large throughout the region 1.3–1.5 R_J), whereas higher-order resonances are substantially narrower. We have confirmed the results described above through a numerical integration of the full equations of motion (see ref. 14).

Planetary ring form and resonances

A recent reanalysis^{10,12} of the Voyager jovian ring images has identified three components to the system (see Fig. 2a). The main ring has an abrupt (<200 km wide) outer boundary whose half-power point is at $1.8086 (\pm 0.0014) R_J$, just outside the

small satellite Adrastea's orbit (semi-major axis $a = 1.8065 R_J$). Its low optical depth ($\tau \approx 10^{-5}$ – 10^{-6}) means that particle-particle collisions are rare^{10,12}. As seen in the back-scattered light that emphasizes large bodies, the main ring is <30 km thick¹⁰. A vertically extended halo, symmetrical about the ring plane, starts exterior to the ill-defined inner edge of the main ring at $\sim 1.71 (\pm 0.01) R_J$, only to disappear somewhere between 1.3 and $1.4 R_J$. The optical depth of the halo is comparable with that of the main ring and the particle size distribution there, as in the main ring, is a power-law starting at a few tenths of a micrometre. The brightness of a newly-discovered 'gossamer' ring^{12,27} decreases linearly with distance from the main ring and has a mean value $\sim 5\%$ that of the main ring. It stretches perhaps as far out as Thebe ($a = 3.15 R_J$) before vanishing from sight; its only notable feature is a $\approx 20\%$ enhancement at R_{syn} .

Because gravitational resonances, as distinct from Lorentz resonances, are so important in defining the Saturn ring structure^{3,4}, we consider their possible connection to the form of Jupiter's ring. Potentially significant gravitational resonances include Amalthea's 5:3 resonances¹¹, which are very near the main jovian ring's outer edge and the orbit of Adrastea: using the formalism of Shu²⁰ with values of J_2 and J_4 given earlier, we locate the horizontal (or eccentricity) resonance at $1.8054 R_J$ and the vertical (or inclination) resonance at $1.8126 R_J$; the mixed resonance is at $1.8071 R_J$. The third of these lies marginally within the positional error of the outer ring edge. The 5:3 resonance is very weak because of the small size of Amalthea coupled with the second-order nature of this resonance; its strength is only $\sim 10^{-5}$ of Mimas's 2:1 resonance which defines the outer edge of Saturn's B ring at the Cassini division, and 3×10^{-5} of Janus's 7:6 resonance which prevents the outer edge of Saturn's A ring from spreading²⁸. Even weaker is Io's 6:1 resonance, which is nearby¹¹. In addition, these resonances are unlikely to be important as one should not expect density waves to be generated, nor the shepherding mechanism to work, in the ethereal Jovian ring¹⁰. More probably, the boundary of the jovian ring is determined by the radial extent to which collisional ejecta is shed from Adrastea¹⁰, before the debris starts to evolve inwards. Nevertheless, the possible connection of this weak resonance with the form of the ring bears close scrutiny during the forthcoming Galileo observations for, if verified, it would require a re-evaluation of the mechanism whereby resonances terminate rings²⁹. For completeness we point out that Callisto's apsidal resonance is located at $1.39 R_J$, in the general vicinity of the inner perimeter of the halo. Note that the embedded satellites Adrastea and Metis have many resonances throughout the main ring.

Lorentz resonances, probably because they act on small grains, seem to account for much of the structure of the jovian ring. The jovian ring halo seems to be bounded by the interior $k = +3$ and $k = +2$ resonances. The outer boundary is easily understood. The orbits of grains in the main ring are expected to evolve rapidly inwards towards the halo because of plasma drag¹⁰. Once particles drift into the resonance zone they receive repeated out-of-plane thrusts and are pushed onto inclined (vertically extended) orbits. All halo particles reside in the region between the $k = +2$ and $k = +3$ resonances (see Fig. 2b); their jumbled trajectories have substantial radial and vertical excursions^{14,15}. The ring may appear to vanish at the strong $k = +2$ resonance because (1) the inward-evolving particles are so widely dispersed, having been scattered by both the $k = +2$ and $k = +3$ resonances, or (2) their extremely large excursions produce collisions into the jovian atmosphere, or (3) the individual particles have by this point simply evanesced through sputtering by magnetospheric particles¹⁰.

As already discussed, the main ring's outer boundary is probably associated with Adrastea, and not with Lorentz resonances. Nevertheless, despite the motion induced by the $k = +1$ and $k = +2$ terms, the overlapping inner $k = +3$ and $k = +4$ resonances produce a deep relative minimum in this locality (see Fig. 2b). The reduced vertical displacements here mean that the ring particles are more densely packed; this may possibly allow them

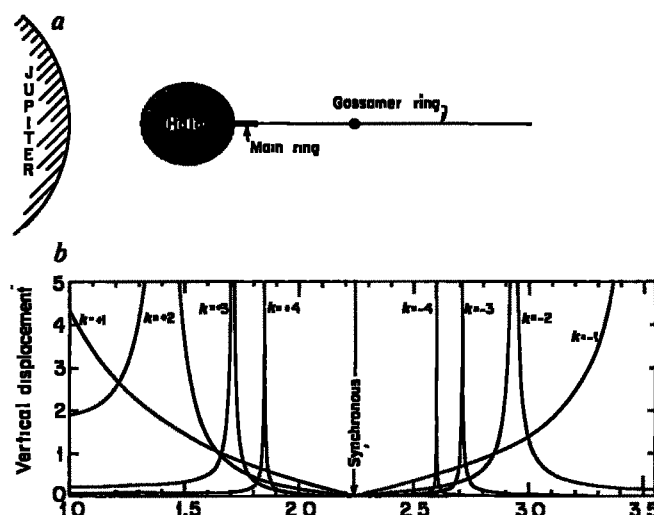


Fig. 2 Jovian ring boundaries are compared with positions of some low-order Lorentz resonances. *a*, A cross-section of Jupiter's ring, as deduced by Showalter^{10,12}. The thin main ring is $\sim 20\times$ as bright as the newly discovered gossamer ring²⁷, whose only feature is a slight enhancement at synchronous orbit ($\odot$). The locations of the outer edge of the gossamer ring and the inner edge of the halo are not well determined. The precise cross-section and density distribution of the halo are poorly constrained. *b*, Responses to the low-order Lorentz force components across the region of the ring in arbitrary units. The $k = +2$ and $+3$ resonances seem to bound the halo, and the $k = -2$ resonance may limit the outer edge of the gossamer ring.

to reach great enough number densities so as to act collectively in shielding themselves from the ambient plasma^{10,12}. Interparticle shielding in a dusty plasma can be such that the average charge on any particle is substantially less than that carried by an isolated grain (whose electric potential would be approximately the plasma's kinetic energy)^{16,17,30}. This reduced charge further lessens vertical motions and augments interparticle shielding¹². Perhaps this process could account for the surprising thinness of the main ring.

The brightness enhancement at R_{syn} in the gossamer ring may have three causes. First, vertical motions resulting from Lorentz forces will be limited in this region, even for the very tiny motes in this ring²⁷, as resonant forces are very weak near R_{syn} where $k \rightarrow \infty$ (see equation (6) and Fig. 2b). For shallow viewing angles such as those used by Voyager, this produces an enhancement at R_{syn} . Second, plasma drag, the principal cause of orbital evolution, vanishes at this point: hence any particles created by micrometeoroid collisions in this region will remain, whereas detritus elsewhere will be continually dragged away from its source region²⁷. Lastly, magnetically dominated particles have a tendency to drift towards synchronous orbit because of systematic charge variations^{16,17} (D. A. Mendix, personal communication).

The $k = -2$ resonance is near where the gossamer ring disappears. Perhaps outwardly-evolving particles are vertically dispersed enough by this resonance to make the already faint ring indistinguishable from background.

Lorentz resonances are much less important for Saturn's rings than for those of Jupiter because strengths are substantially lower (Table 1): Saturn's magnetic field is weaker as well as more regular than Jupiter's, and relative velocities are generally less because the ring straddles R_{syn} and the orbital velocity at R_{syn} is lower. In addition, saturnian ring particles are usually much larger (except for restricted regions^{3,4,10}) and charges smaller³⁰ so that charge-to-mass ratios are low. Furthermore, in the main ring system, optical depths are high so that our calculations, which ignore collisions, are moot.

The (interior) $k = +4$ resonance for Saturn is close to the B/C ring boundary ($1.525 R_S$) whereas the (exterior) $k = -3$ resonance lies just within the A ring edge ($2.267 R_S$) and the Keeler

gap ($2.263 R_n$). The (inner) $k=+4$ resonance is close to the gravito-magnetic stability limit for charged, massless grains launched with Keplerian velocity at $1.525 R_n$ (ref. 31) and the place where the magnetic equilibrium surface becomes undefined at $1.56 R_n$ (ref. 32); these apply, however, to particles for which q/m is effectively infinite.

It seems then that some part of the jovian ring's form is dictated by a resonance phenomena, albeit one different than the usual gravitational resonance of celestial mechanics. The

new resonance mechanism described here acts on small charged grains when they orbit through a non-uniform magnetic field. Additional study of the nature of this resonance will advance our understanding of the operation of all resonances.

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Strontium isotope evidence for preserved density stratification in the main zone of the Bushveld Complex, South Africa

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The Bushveld Complex formed from two isotopically distinct magmas. Magma addition and mixing can be tracked by measuring initial strontium isotope ratios of cumulates. Rocks with ratios of 0.7065 lie below the lower 2.2 km of the main zone (ratio = 0.7085), whereas those with ratios of 0.7073 lie above it. This unit represents a liquid layer intruded into the density-stratified magma pile near the base of the chamber. The Merensky Reef was produced by mixing of the pile with underlying trapped liquid enriched in precious metals.

EVIDENCE is accumulating that the layered rocks of the Bushveld Complex are the products of fractional crystallization from two magma series and their mixtures¹⁻⁵. Field and laboratory investigations have helped detect early derivatives of these series in the suites of quenched marginal rocks and related sills. The end-members have yet to be discovered. The stratigraphically older series were derived from SiO₂- and MgO-rich magmas similar to olivine boninites⁶; rocks of the other series are tholeiites. The lineages are termed U and A because of their ultramafic (orthopyroxene) and anorthositic (plagioclase) rock products, respectively⁷. U magmas are represented by quenched micro-orthopyroxenites, rich in incompatible elements (Zr = 70-300 p.p.m., Rb = 20-50 p.p.m.), 900-1,600 p.p.m. Cr and initial Sr ratio (R_0 ; ⁸⁷Sr/⁸⁶Sr₂₀₅₀) of 0.703-0.705 (refs 8, 9; Table 1). The marginal rocks quenched from magmas with a large A component are two-pyroxene microgabbros with low contents of incompatible elements (Zr ~ 15, Rb ~ 2 p.p.m.), ~250 p.p.m. Cr and R_0 values of 0.7065-0.7077. Rocks with compositions intermediate between these two 'end-members' are most abundant along the margin of the upper critical zone (Fig. 1) and are petrographically microgabbroic.

It is important to understand the mechanism of addition of parental magmas to the Bushveld chamber, because recent hypotheses concerned with the origin of layered complexes draw heavily on density stratification as a possible means by which

layering can be generated. Competing, yet (in many cases) overlapping hypotheses demand attention. Irvine and co-workers^{7,10} propose that rock layers grow laterally from the walls of magma chambers at shallow angles. The composition of each rock unit is controlled by that of its parental liquid layer, which forms part of a density-stratified system consisting of many such layers. Thus, new magmas may be intruded into stratified chambers at levels consistent with their density. Another school of thought¹¹ believes that such intrusion may occur as a turbulent plume which entrains existing magmas, spreads out in a discrete layer and controls sulphide precipitation. Most other hypotheses (see refs 12, 13 and A. J. Naldrett *et al.*, manuscript in preparation) depend on the new addition of magma forming a separate layer within a developing intrusion, at some level where a stratified magma system would have an appropriate density to accommodate the new liquid.

Unfortunately, definitive evidence in support of mechanisms such as density stratification, double-diffusive convection, turbulent plumes or other fluid-dynamic hypotheses in magma chambers has not been available. Evidence for the first of these possibilities is lacking because liquid layers are thought to be quickly destroyed during the processes of fractional crystallization and mixing, especially if they are thin as has mostly been assumed. This article presents field, geochemical and isotope evidence from the eastern Bushveld Complex for a 'fossilized'

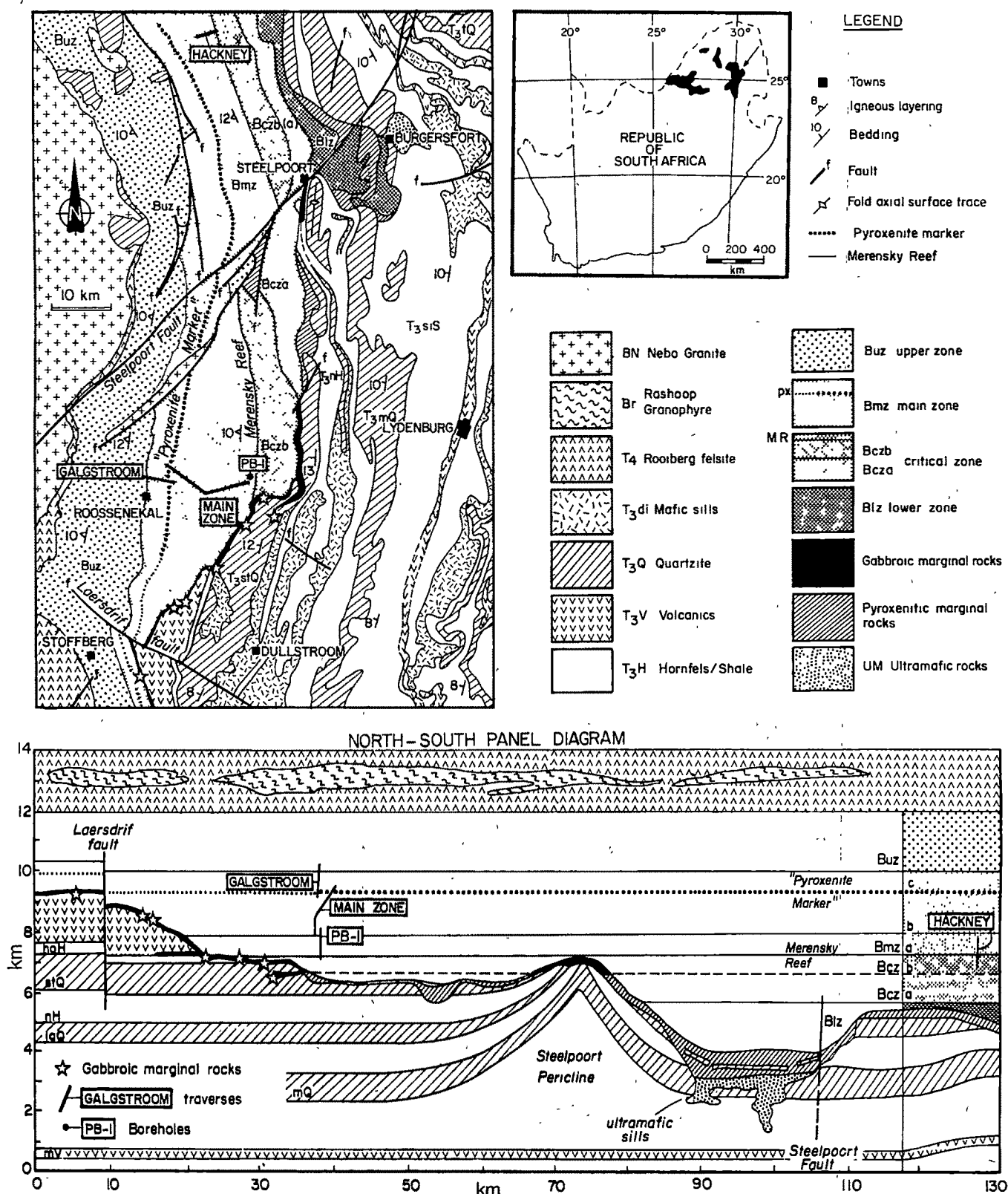


Fig. 1 Map of a portion of the eastern Bushveld Complex with a panel diagram from south to north along the contact region and stratigraphic column of the Bushveld Complex and Transvaal Sequence. Positions of sample traverses and gabbroic marginal rocks are annotated. Codes for lithologies follow ref. 1.

2-3-km thick layer of gabbroic magma within the 9-km thickness of layered rocks, which differentiated *in situ* with relatively minor mixing between the under- and overlying magma columns.

Geochemistry

Three hundred and fifty cumulate rock samples were collected from the eastern Transvaal and analysed by X-ray fluorescence

for major elements and 19 trace elements as part of a comprehensive study of the layered rocks of the eastern Bushveld Complex (see ref. 1 for analytical procedures). Fifty-five of these samples were analysed for Sr isotopes. Samples were selected to provide ample vertical coverage of the layered sequence, representative collections from each zone or group of petrographically related rocks and detailed coverage across important boundaries where

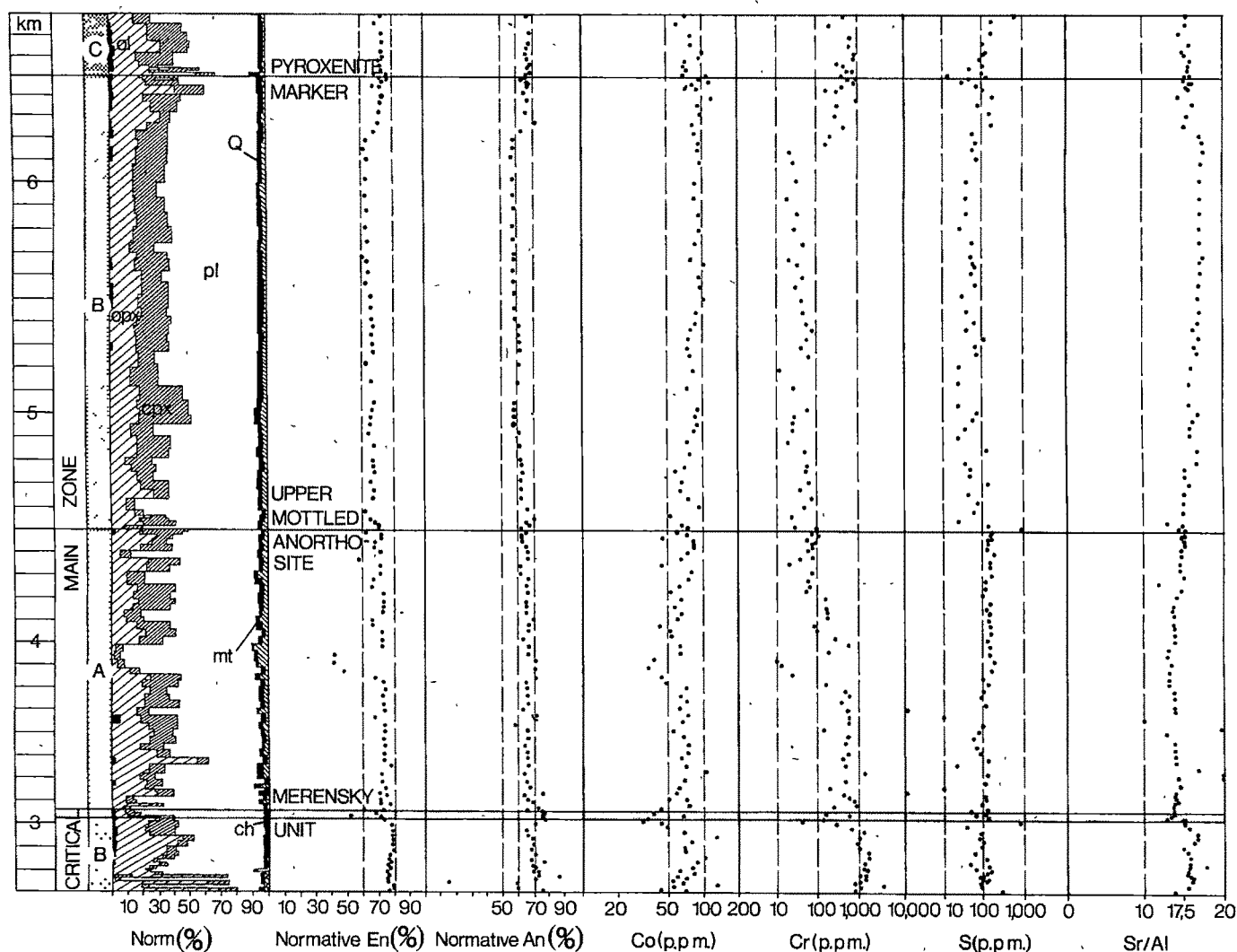


Fig. 2 Whole-rock geochemical data from 300 m below the Merensky Reef in subzone B of the critical zone to 300 m above the pyroxenite marker in subzone C of the main zone. Normative cumulus minerals: ch, chromite; mt, magnetite (above Merensky Reef); cpx, clinopyroxene; opx, orthopyroxene; pl, plagioclase; ol, olivine (Fe-rich); Q, quartz.

there are major changes in the cumulate parageneses. Thirty marginal rocks spanning the lower 5.5 km of the complex were also analysed (N. M. Evensen, B. V. Rao and M. R. S., in preparation). Concentrations of Rb and Sr were determined by isotope dilution.

The region of greatest interest lies between the Merensky Reef and the 'pyroxenite marker'^{14,15}, and comprises subzones A and B of the main zone (Fig. 2). In many respects, the main zone is an anachronism in the evolution of the complex. Its relatively homogeneous, two-pyroxene gabbros contrast in composition and lithology with the strongly layered units of the upper critical zone and the equally well-layered upper zone, which lie below and above, respectively. In the critical zone, the platinum group element (PGE) tenor of mineralized layers increases upwards, to climax in the UG2 chromitite layer and the Merensky Reef (Fig. 1). In the upper zone, abundant cumulus magnetite precipitated along with orthopyroxene, clinopyroxene and plagioclase, with Fe-rich olivine occurring at later stages. Whole-rock samples beneath the Main Magnetite Layer contain up to 5.6 p.p.m. total PGE¹⁶, with an average concentration of ~1.3 p.p.m.¹⁷.

Plots of norms, mineral compositions, some trace elements and Sr/Al ratio against stratigraphic height for subzones A and B of the main zone show major breaks at the base and top of this interval (Fig. 2). Discontinuities in the Pt/(Pt+Pd) ratio at the same levels were interpreted as evidence for addition of new magma¹⁷. At the pyroxenite marker the intruding liquid was 'replenished' in PGE, because the main zone is relatively dep-

leted compared with the critical and upper zones. The marker was originally considered as the expression of addition of fresh magma^{14,15}, even though supporting field relations like cross-cutting layers and footwall disturbances are ill-exposed or lacking in the eastern Bushveld. At the level of the Merensky Reef, however, there is much field^{1,9} and geochemical^{8,18} evidence for intrusion of new magma.

The lower 200 m of the main zone is lithologically variable. In the eastern Bushveld, the En content of orthopyroxene increases by up to 15 mol % in this interval (Fig. 2). Upward trends are fairly smooth, suggesting that simple fractional crystallization, with perhaps local addition of fresh magma (such as at the upper mottled anorthosite, Fig. 2) gave rise to gradually evolving profiles. Differentiation trends begin a marked reversal 300–500 m below the pyroxenite marker, but are most pronounced within metres of this layer. Other compositional tracers mirror these paths. Were subzones A and B of the main zone to be removed from the Bushveld Complex, it appears that the post-Merensky Reef and sub-pyroxenite marker compositions would prescribe a continuous trend, with only minor discontinuities between their whole-rock compositions. I suggest that this packet is an aberration on the normal fractionation sequence of critical zone to subzone C of the main zone and then upper zone. The petrographic and whole-rock composition suggests that the packet is a thick layer of liquid A that was intruded into the Bushveld chamber near its base, which has been preserved simply because of its thickness. However, mixing did occur at its lower and upper boundaries.

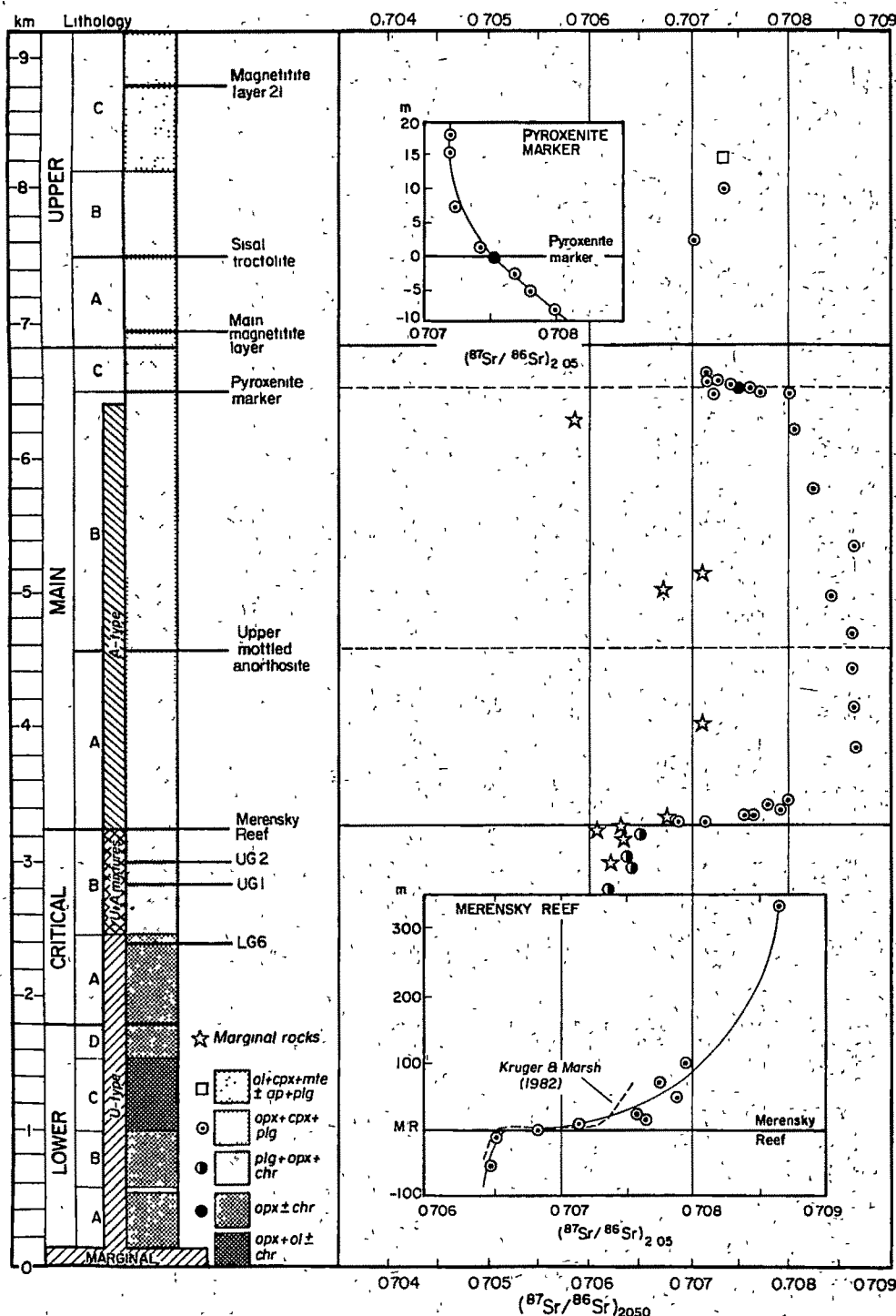


Fig. 3 $^{87}\text{Sr}/^{86}\text{Sr}$ at 2,050 Myr against stratigraphic height for the traverses and marginal rocks detailed in Fig. 1. Enlarged boxes show detail at the Merensky Reef and pyroxenite marker. Italicized mineral names are at levels of their addition (+) or deletion (-) from the cumulus assemblage.

Strontium isotope systematics

The Rb/Sr isotope age of the Bushveld layered rocks is 2,050 Myr. Early work gave an age of $1,954 \pm 30$ Myr on 13 samples²⁰, and Hamilton⁸ reported an average age of $2,095 \pm 24$ Myr. Both used the ^{87}Rb decay constant of $1.39 \times 10^{-11} \text{ yr}^{-1}$. Re-calculation of Hamilton's data with $\lambda^{87}\text{Rb} = 1.42 \times 10^{-11} \text{ yr}^{-1}$ gives an age of 2,050 Myr⁹. Unfortunately, because two magma types of differing R_0 are represented in the layered rocks, average ages such as these are less relevant than data from units of layered rocks which were derived from one or other of the magma types, or a stable mixture between them. Isochron ages of 2,048–2,057 Myr were acquired from sample sets from the lower zone, subzones A and B of the main zone, and the upper zone (M.R.S. *et al.*, in preparation). A neodymium age of $2,055 \pm 155$ Myr is reported by M.R.S. *et al.*, (in preparation). It seems that any Rb and Sr mobility must have operated homogeneously over cubic kilometre scales and is thus unlikely. The age of the

complex is also constrained by isotope age-dating of rock units from the Transvaal Sequence into which the complex is intruded, and the Bushveld granite, which cuts the layered rocks. The most recent data have been compiled by von Gruenewaldt *et al.*¹⁹.

All Bushveld marginal rocks have R_0 values higher than expected from normal mantle (Rb/Sr = 0.03) at 2,050 Myr; the values range from 0.704 (U > A) to 0.7077 (A > U). Layered rocks have a wider range (0.704–0.7086). Such high values might originate in various ways, such as contamination on local or regional scales or Rb mantle enrichment. A brief treatment of Bushveld magma evolution is described here. Contamination of magmas has been proposed on the bases of trace element and isotope data^{1,3,8,9,20}. Because the R_0 ranges of marginal rocks, and cumulates are approximately the same, local contamination of magmas in the chamber after quenching of the marginal rocks is considered unlikely. Regional contamination, however, is more probable, especially for the main zone, where values up

Table 1 Representative analyses of marginal rocks

	CO-211	CO-114	CO-071	CO-073
	Blz	Bcza	Bczb	Bmz
	U _{1a}	U > A	A > U	A ₁
SiO ₂	55.48	57.01	52.42	49.92
TiO ₂	0.28	0.40	0.79	0.35
Al ₂ O ₃	10.48	12.81	15.56	16.37
FeO	9.25	9.36	10.64	9.85
MnO	0.17	0.17	0.19	0.19
MgO	15.42	10.29	6.67	8.22
CaO	6.10	6.72	10.20	13.17
Na ₂ O	1.73	1.75	2.97	1.74
K ₂ O	0.76	1.23	0.32	0.11
P ₂ O ₅	0.05	0.09	0.15	0.01
Cr ₂ O ₃	0.22	0.11	0.04	0.04
NiO	0.06	0.05	0.04	0.03
(p.p.m.)				
Cr	1,349	765	297	246
Ni	403	218	111	135
Rb	30.7	47.3	4.17	1.69
Sr	159	199	247	323
Cu	60	63	29	13
Zr	236	105	137	12
Y	32	16	30	10
Eu*	0.2	-1.8	-5.4	1.7
Ce _N	30.3	48.2	44.6	4.4
Ce _N /Yb _N	7.4	9.1	3.9	1.2
En _{opx}	73.0	85.6	54.9	63.5
An _{plg}	61.2	ND	52.0	59.0
R ₀ at 2,050 Myr	0.70303 ± 6	0.70587 ± 9	0.70633 ± 4	0.70680 ± 4

Samples CO-211 and CO-073 are close to the end-member compositions, CO-114 is a fractionated derivative of U mixed with high- R_0 A liquid; CO-071 is A mixed with some fractionated liquid U. Analyses summed to 100% anhydrous; all Fe as FeO; ND, not determined; Eu*, chondrite-normalized Eu anomaly; R_0 , ($^{87}\text{Sr}/^{86}\text{Sr}$)_{2,050} (ref. 9 and M.R.S. *et al.*, in preparation); rare-earth element data from M.R.S. and M. P. Gorton (unpublished data). Blz, adjacent to lower zone; Bcza, adjacent to lower critical zone; Bczb, adjacent to upper critical zone; Bmz, adjacent to main zone.

to 0.7086 exceed those of possible local contaminants like the Rooiberg felsites ($R_0 < 0.708$; R. E. Harmer, personal communication). However, Pretoria Group shales²¹ have initial ratios between 0.708 and 0.723 (ref. 9) and so may be more likely candidates. Combined Nd and Sr isotope data (M.R.S. *et al.*, in preparation) indicate that both U and A magmas lie in the enriched quadrant of a $\epsilon_{\text{Nd}}/\epsilon_{\text{Sr}}$ plot. The U dominant rocks lie closer to the "Bulk Earth" composition, but increasing amounts of A drives points diagonally from the fulcrum towards $\epsilon_{\text{Sr}} = 150$, $\epsilon_{\text{Nd}} = -20$, typical of crustal material²². Thus, relatively homogeneous contamination on scales of hundreds of thousands of cubic kilometres is indicated.

The addition of liquid A to the Bushveld magma chamber has been monitored by making use of the widely contrasting R_0 values of the U and A magma series (ref. 9 and M.R.S. *et al.*, in preparation). The Sr isotope data provide confirmation of the unusual character of the main zone (Fig. 3). R_0 values of cumulates increase from 0.7065 immediately below the Merensky Reef to 0.7085 about 200 m into subzone A of the main zone. The increase in R_0 across the Merensky Unit is sudden. It was first noted by Kruger and Marsh¹⁸ and equated with an addition of new magma with high R_0 . Geochemical parameters through the first 2.2 km of the main zone (Fig. 2) prescribe a trend suggestive of steadily increasing fractionation, on which broad cyclic sweeps are superimposed, particularly evident in the Co data. Samples from subzone A of the main zone have constant R_0 values, regardless of the irregularities in the trends. In subzone B, which has somewhat more regular data for Co, the isotope data show local decreases. I do not dismiss the possibility, however, that high R_0 magma was added intermittently within the main zone. In the limitations of sample

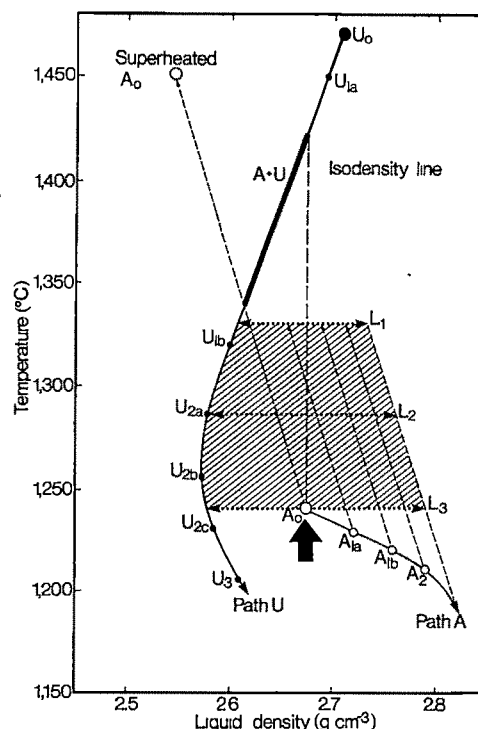


Fig. 4 Variation in liquid density in relation to temperature for Bushveld parental magmas at 5 kbar. The range of densities expected in the chamber are controlled by initial densities (points U_0 and A_0), upon which are superimposed the effects of fractional crystallization of different minerals. The crystallization of orthopyroxene from U liquids along path (U_0-U_3 , where subscript refers to derivative type) will decrease liquid density until plagioclase begins to crystallize (near U_2), whereupon density increases, assisted by late-stage iron enrichment. A liquids increase in density with temperature drop during plagioclase crystallization (path A). At 1,330 °C (CO-114 liquidus temperature at 1 atm⁵ + 10 °C kbar⁻¹) the chamber might be expected to contain liquids with a wide range of densities, comprising U derivatives, superheated A derivatives and A + U mixtures, located along line L_1 . Further temperature drop will increase that range through L_2 to L_3 . New liquids could readily be accommodated at levels controlled by their densities, like at point A_0 , in the middle of the density range (solid arrow). Thick line A+U represents the temperature/density range over which U derivatives and superheated A derivatives might finger mix; thin dashed lines are superheating paths for A derivatives.

coverage, values of R_0 remain approximately steady through the bulk of the main zone, although slight fluctuations do begin 300–500 m below the pyroxenite marker, where other indices also become perturbed. At the same distance below the marker, R_0 decreases to 0.708, but the most marked decrease, to 0.7073, occurs within a 25-m interval centred on the marker. The R_0 remains at ~0.7073 through the rest of the complex.

These data show that the main zone represents a slab of layered cumulates that were derived from high R_0 liquid. Its basal portions mixed with the underlying post-Merensky Reef U + A residuum to produce a steep R_0 gradient. Similarly, the less rapid R_0 drop at the top of subzone B can be equated with deeper penetration of the front along which A and A + U magmas mixed. Possibly the pyroxenite marker might be considered as a sort of semipermeable membrane along the interface between 'old' and 'new' magmas. The post-Merensky Reef and pyroxenite marker R_0 values are almost contiguous: the difference of 0.0005 between the 0.7065 value at the Merensky Reef and 0.707 at the pyroxenite marker may be ascribed to upward transfer of some A residuum from the crystallizing subzones A and B of the main zone.

Mass-balance calculations can be made to determine U:A ratios at the time of formation of the Merensky Reef and the upper zone, assuming no local contamination further affected

Table 2 Rb, Sr and isotope data in relation to stratigraphic height

Sample cumulates	Height above M. Reef (m)	Rb	Sr	$^{87}\text{Rb}/^{86}\text{Sr}$	$^{87}\text{Sr}/^{86}\text{Sr}$	$(^{87}\text{Sr}/^{86}\text{Sr})_{2050}$
BDS-120	5,040	1.11	281.1	0.0114	0.70766 $\pm$ 4	0.70732
BDS-87	4,740	4.00	292.5	0.0396	0.70856 $\pm$ 8	0.70739
BDS-82	4,450	1.94	205.4	0.0273	0.70785 $\pm$ 5	0.70704
BDS-124	4,140	19.02	447.8	0.1229	0.71104 $\pm$ 2	0.70741
BDS-54	3,860	1.80	400.0	0.0130	0.70786 $\pm$ 6	0.70748
BDS-62	3,258	4.31	214.0	0.0583	0.70891 $\pm$ 3	0.70719
BDS-69	3,255	1.54	241.7	0.0184	0.70774 $\pm$ 5	0.70720
BDS-68	3,248	2.11	247.3	0.0247	0.70794 $\pm$ 3	0.70721
BDS-67	3,241	2.94	331.7	0.0256	0.70819 $\pm$ 5	0.70743
BDS-66	3,240	4.82	96.5	0.1446	0.71180 $\pm$ 2	0.70753
BDS-65	3,238	2.51	314.6	0.0231	0.70839 $\pm$ 3	0.70771
BDS-64	3,235	1.83	267.5	0.0198	0.70837 $\pm$ 5	0.70779
BDS-63	3,232	1.84	309.1	0.0172	0.70851 $\pm$ 5	0.70800
BDS-30	3,140	2.42	256.5	0.0273	0.70808 $\pm$ 3	0.70727
BDS-35	2,940	1.87	280.9	0.0193	0.70881 $\pm$ 3	0.70824
BDS-41	2,530	3.06	289.2	0.0306	0.70911 $\pm$ 2	0.70821
BDS-09	2,140	3.18	296.3	0.0311	0.70953 $\pm$ 6	0.70861
BDS-17	1,690	7.15	321.1	0.0644	0.70994 $\pm$ 6	0.70804
BDS-49	1,410	8.42	275.5	0.0885	0.71123 $\pm$ 4	0.70862
PB1-383	1,220	6.97	270.9	0.0745	0.71077 $\pm$ 2	0.70857
PB1-526	840	5.79	219.9	0.0762	0.71090 $\pm$ 2	0.70865
PB1-953	330	5.77	235.5	0.0709	0.71075 $\pm$ 3	0.70866
BDS-216	100	1.76	284.9	0.0179	0.70845 $\pm$ 3	0.70792
PB1-1256	70	9.57	342.3	0.0809	0.71013 $\pm$ 3	0.70774
BDS-219	50	1.81	364.3	0.0144	0.70832 $\pm$ 6	0.70790
PB1-1282	25	2.31	349.9	0.0191	0.70816 $\pm$ 3	0.70760
BDS-218	18	5.24	279.1	0.0543	0.70926 $\pm$ 6	0.70766
PB1-1319	4	3.97	380.2	0.0302	0.70802 $\pm$ 3	0.70713
PB1-1332	0	2.08	375.0	0.0160	0.70731 $\pm$ 3	0.70684
BDS-220	-15	0.73	305.4	0.0069	0.70761 $\pm$ 5	0.70651
BDS-202	-55	0.53	293.8	0.0052	0.70665 $\pm$ 2	0.70650
MDH5-202	-320	1.39	373.3	0.0108	0.70672 $\pm$ 3	0.70640
MDH24-79.1	-500	0.55	324.6	0.0049	0.70634 $\pm$ 2	0.70620
Marginal rocks						
CO-059	3,090	15.65	353.2	0.1282	0.70964 $\pm$ 7	0.70585
CO-052	1,800	1.76	405.2	0.0126	0.70754 $\pm$ 5	0.70717
CO-048	1,740	4.06	338.2	0.0347	0.70773 $\pm$ 2	0.70670
CO-073	~0	1.69	340.4	0.0144	0.70722 $\pm$ 4	0.70680
CO-250	~0	3.22	341.5	0.0273	0.70710 $\pm$ 2	0.70629
CO-251	~0	4.41	325.4	0.0392	0.70720 $\pm$ 1	0.70604
CO-071	-40	4.17	273.8	0.0441	0.70763 $\pm$ 4	0.70633
CO-089	-360	2.13	352.0	0.0175	0.70667 $\pm$ 2	0.70615

$^{87}\text{Sr}/^{86}\text{Sr}$ ratios are given at the 1 σ level. Analyses of NBS-987 are reported as 0.71026 $\pm$ 3 (1 σ). Boxed samples: Merensky Reef (PB1-1332) and pyroxenite marker (BDS-66).

the regionally contaminated magmas. The crystallization rate and bulk D of the rocks below the Merensky Reef cannot be estimated at present without excessive speculation. It is assumed that U liquids had $R_0 \sim 0.704$ and Sr = 167 p.p.m. (sample CO-211; ref. 9), quenched liquids at the top of the critical zone have $R_0 = 0.7065$ and Sr = 330 p.p.m. (CO-250; ref. 9) and quenched liquids from which the upper zone crystallized had $R_0 = 0.707$ with Sr = 380 p.p.m. (CO-052). The pure A liquids (main zone) had $R_0 \sim 0.7085$ and high Sr (>450 p.p.m.). Using the equations of De Paolo²³, adapted for simple mixing, it can be calculated that the Merensky Reef crystallized from a total mixture of 88% U + 12% A. A further 25% of fractionated A residuum added to the elevated Merensky Reef source magma simulates the R_0 of the upper zone, which crystallized from a total U:A mixture of 71:29.

Isotope relations

Cumulates of subzones A and B in the main zone have $R_0 \sim 0.7085$ (Fig. 3). The marginal rocks that occur along the basal contact of the main zone (Fig. 1) have $R_0 = 0.706\text{--}0.7075$ (ref. 9). The isotope ratios of marginal and main zone layered rocks are completely out of equilibrium. The marginal rock ratios closely resemble those of layered rocks from the upper critical zone (0.7061–0.7065; Table 2) and the upper zone (0.7073). They

are probably the quenched equivalents of the magma(s) that occupied the chamber at Merensky Reef times. Derivatives of these magmas, modified at a later stage by addition of main zone residual liquids, crystallized rocks that now occur ~3 km higher in the succession.

The marginal rocks along the basal contact of the main zone occur as a thin tenuous swath, some 10% of the extent of the upper critical zone marginal zone. Field relations⁹ suggest that the intrusion of A liquids that were to give rise to the main zone practically destroyed the original marginal rocks and replated the basal margin with some high- R_0 material. Two marginal rock generations occur south of the Laersdrif Fault (Fig. 1), where magnetite-rich microgabbros are intruded by fine-grained, two-pyroxene gabbro sheets (J. Schweitzer, personal communication).

Density considerations

The lower subzones of the main zone crystallized as a sheet from A liquids that were added in copious amounts to the Bushveld magma chamber, which was, at the time, crystallizing the upper critical zone from U >> A liquids. The massive addition of A liquid occurred near the base of the chamber, at a level consistent with its density. The magmas in the chamber were relatively hot yet dense; A magmas were cooler and slightly less

dense. The A sheet underwent limited mixing at its base with fractionated U+A mixture, giving rise to the steep R_0 gradient. The fact that the Merensky Reef is located at this level is surely more than coincidence, even though precious metals were steadily concentrated in residual magmas during formation of the lower and critical zones²⁴, as also shown by upward increases in the PGE tenor of the chromitite layers. I argue that evolution of the Merensky Reef is inextricably bound to the addition of A magma.

I performed melting experiments on rocks considered to be quenched $U \gg A$ (CO-114) and $A \gg U$ (CO-071) mixtures⁵ (Table 1). Their liquidus temperatures were 1,275 and 1,210 °C, respectively. The amount of chromite precipitated on mixing two such magmas depends on their proportions and differences in fO_2 (ref. 5). Because the critical zone contains abundant chromitite layers up to 1.6 m thick and mineral compositional variations across such layers are relatively minor (C. J. Hatton, unpublished data), it can be assumed that the volumes of magma A intermittently added to the magma chamber were small (supported by the isotope data), but their oxygen fugacities were very different. The pre-Merensky Reef rocks retain evidence of continuous sulphur saturation of the parental magmas²⁴, yet sulphides in the main zone are extremely rare. The bulk compositions of A and U magmas were dissimilar. Addition of 2–3 km of magma A would have increased pressures at the top of the critical zone by ~1 kbar. I suggest that the changes in T , P , X , fO_2 and fS_2 imposed on the trapped packet of U+A magma mixtures from which the upper critical zone was crystallizing were sufficient to stimulate the rapid precipitation of olivine and sulphides rich in precious metals, so giving rise to the Merensky Reef.

At the top of the A sheet, the cool dense A was overlain by hotter, less dense 88% U+12% A, stable conditions ideally suitable for diffusive mixing. Less dense fractionated residual from crystallization of the A sheet were added to the overlying magma column, causing the downward migration of the interface controlled simply by volume changes and mass diffusion. The liquids lying above the pyroxenite marker were homogenized to $R_0 = 0.7073$. Perhaps the abundant upper zone magnetite (~10 modal %) is a signature of the upward transfer of Fe-enriched residual material.

Density relations of some members of the U and A families have been investigated²⁵. Figure 4 represents conditions in the magma chamber at 5 kbar pressure²⁶ before intrusion of A liquids. Liquids with different densities would be present, density contrasts having a greater range with falling temperature. New A_0 liquid or its derivatives could intrude readily the stratified pile at a level controlled by its density, and elevate the superincumbent liquid column, still enriched in precious metals, by 2–3 km.

Conclusions

Subzones A and B of the eastern Bushveld Complex represent a frozen interval in its evolution, where a massive inflow of gabbroic liquid into the magma chamber, at a level consistent with its density, split the crystallizing column of U+A liquids. Figure 5 describes the strontium isotope evolution of the eastern Bushveld Complex by a scheme of magma addition and mixing. In simple terms, the elevation of the post-Merensky Reef residuum gave rise, with later mixing at the top of the A sheet, to the pyroxenite marker, subzone C of the main zone and the upper zone. Mixing at the base of the sheet was over a 200–300-m interval, which created a pronounced R_0 gradient, along with lithological and compositional fluctuations. Marginal rocks with isotope ratios out of equilibrium with adjoining cumulates attest to this intrusion mechanism. Minor addition of A liquids occurred in the crystallizing sheet. Thus, the magma generation and intrusion chronology of the Bushveld Complex is simplified and Occam's razor rediscovered.

This is the first evidence of fossilized density stratification in a large layered intrusion. It provides strong evidence that double-diffusive convection and magma mixing were important processes in the evolution of the Bushveld Complex.

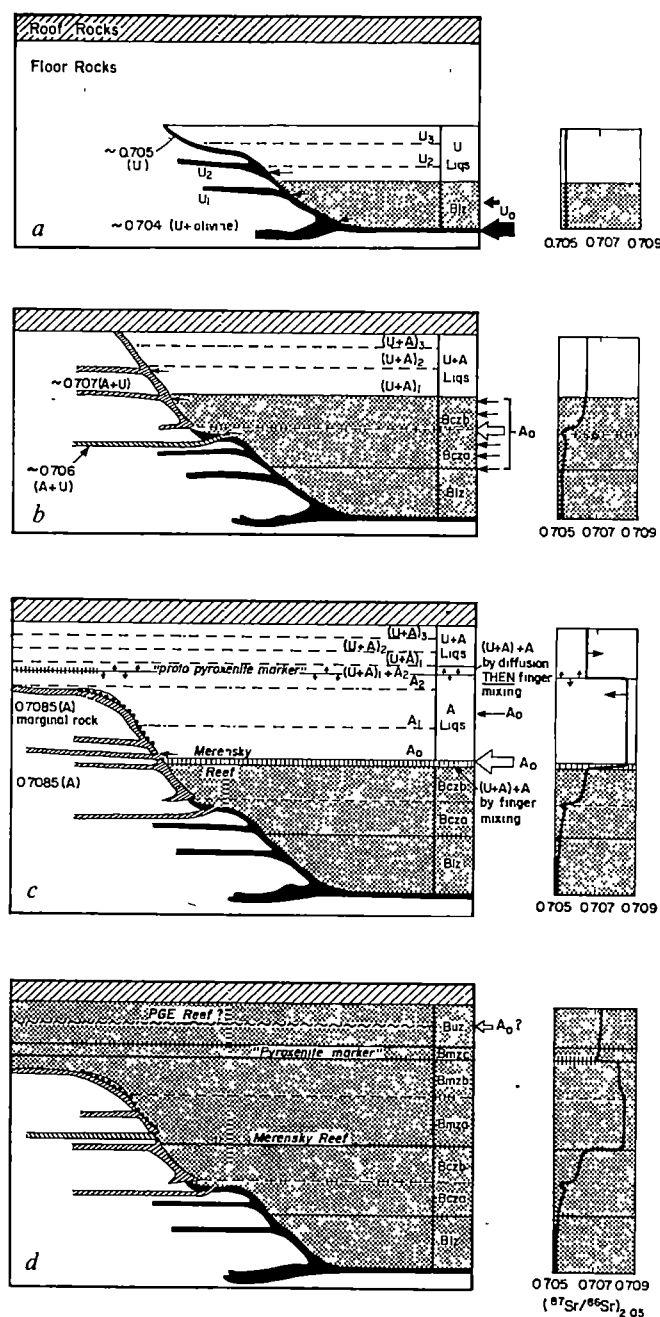


Fig. 5 Diagram of the possible sequence of events that gave rise to the Sr isotope profile of the eastern Bushveld complex (M.R.S. *et al.*, in preparation). *a*, U₀ magmas are intruded into the chamber and differentiate, giving cumulates, U derivatives and related sills with primitive R_0 (0.703–0.704). *b*, Addition of A₀ liquids commences during lower critical zone times, with major addition at subzone A–B boundary. R_0 in cumulates and residual U >> A mixtures increases steadily to 0.7065. Sills of U+A magmas are intruded into the floor rocks. *c*, Massive addition of A₀ occurs near the base of the chamber and splits the U+A liquid pile. Mixing with basal trapped liquid makes the Merensky Reef. The proto-pyroxenite marker forms. The R_0 of precipitating cumulates increases dramatically to 0.7085. *d*, Crystallization of the A sheet continues. Transfer across the proto-pyroxenite marker decreases in cumulates of subzone B to 0.708 and increases R_0 in subzone C of the main zone and the upper zone to 0.7073.

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Identification of serotonin M-receptor subtypes and their specific blockade by a new class of drugs

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We describe a new class of drugs that selectively block serotonin M-receptors on peripheral neurones. Because of their high affinity, some of these drugs are the most potent of any pharmacological class yet reported. They have allowed the identification of three M-receptor subtypes, one of which is responsible for mediating the painful effects of serotonin in humans.

GADDUM and co-workers suggested the existence of different serotonin (5-hydroxytryptamine, 5-HT) receptor subtypes when they showed that this substance contracts the guinea pig ileum by two different mechanisms: a direct action on the smooth muscle and an indirect action mediated by the release of acetylcholine from parasympathetic nerve endings. Because the former mechanism was blocked by dibenzylamine (D) and the latter by morphine (M), the excitatory 5-HT receptors on smooth muscle and parasympathetic nerve endings were designated D- and M-receptors respectively^{1,2}. Neither dibenzylamine nor morphine is selective or potent in its antagonism of 5-HT. In fact morphine prevents the indirect contractile action of 5-HT in this preparation not by a specific blockade of neuronally located receptors, but by a non-selective inhibition of acetylcholine release^{3,4}.

Although many potent new 5-HT antagonists for smooth muscle D-receptors have subsequently become available, they are all completely inactive at M-receptors located on peripheral neurones. Some structural analogues of (-)-cocaine, which inhibits the neuronal actions of 5-HT on the guinea pig ileum^{1,2,5} and sympathetic ganglia^{6,7}, and metoclopramide behave as competitive antagonists at these sites⁸⁻¹¹. These compounds, however, have several other pharmacological properties and display only limited potency and selectivity for M-receptors^{10,12,13}. Thus, despite the wide distribution of excitatory 5-HT receptors in the peripheral nervous system¹⁴, their pharmacological and functional characterization has been greatly hindered by the lack of potent, selective agonists and antagonists. We have adopted an approach analogous to that originally used for generating selective antagonists for β -adrenoreceptors and histamine H₂-receptors: the naturally occurring ligand, in this case 5-HT, was used as the starting point for chemical derivation¹⁵⁻¹⁷. Highly selective agonists for either D- or M-receptors were synthesized and extremely potent and selective antagonists of

neuronal 5-HT M-receptors were produced. Large differences in the affinity constants obtained for these antagonists on different isolated tissues containing neuronal excitatory 5-HT receptors show that there are at least three pharmacologically distinguishable M-receptor subtypes. Preliminary reports of some of these findings have been reported elsewhere¹⁸.

Assay systems

Quantitative responses to the newly synthesized 5-HT analogues were routinely obtained in six different *in vitro* assay systems. A minimum of three independent experiments were conducted for each compound and complete concentration-response curves were constructed. To assess their affinity for M-receptors three bioassays were used: the de-sheathed rabbit vagus nerve, the isolated rabbit heart, and the guinea pig ileum incubated in the presence of 10⁻⁶ M methysergide (see Figs 1, 2 legends). In the first two assay systems 5-HT D-receptor antagonists, such as methysergide (10⁻⁵ M, *n* = 3, data not shown), have no influence on the action of 5-HT and in the third assay, using guinea pig ileum, 10⁻⁶ M methysergide is continuously present to abolish the direct action of 5-HT on the smooth muscle D-receptors present in this preparation. We also used three non-M-receptor systems. Contractile responses of rat uterine strips were used to assess the affinity of compounds to smooth muscle 5-HT D-receptors. In addition, displacement of the specific binding of [³H]5-HT or 2-[¹²⁵I]doxylsergic acid diethylamide ([¹²⁵I]LSD) from rat cortex membranes was used for measuring the affinity of compounds to central 5-HT₁ and 5-HT₂ receptors, respectively^{19,20}.

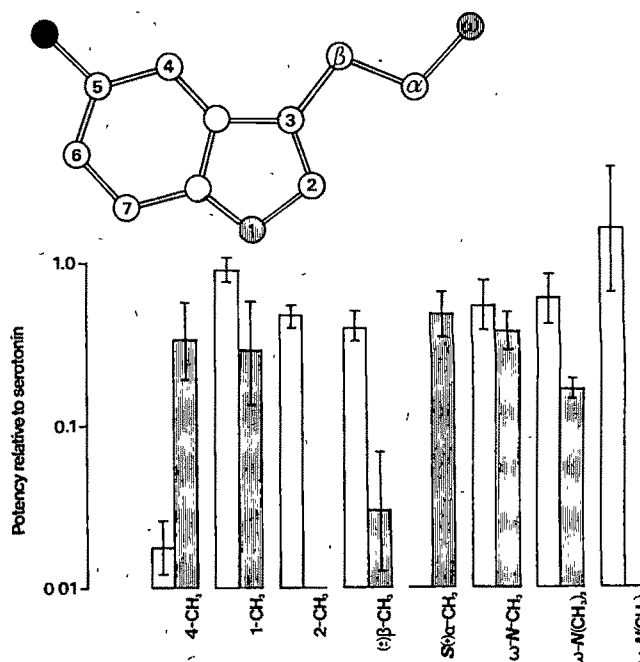
Selective agonists for M- and D-receptors

Our initial chemical programme was based on the assumption that different conformations of the 5-HT molecule are required for activation of M- and D-receptors. By systematic methyl substitutions in the indole nucleus and ethylamine side chain we attempted to reduce the conformational freedom of the 5-HT

* Deceased

Fig. 1 Agonist potency of methylated 5-HT analogues relative to 5-HT (potency = 1.0) on the de-sheathed rabbit vagus nerve (open columns) and the rat uterus (striped columns). Each column indicates the geometric mean value obtained from at least three independent experiments. Vertical lines on each column indicate 90% confidence limits.

Methods. Isolated rabbit vagus nerve. New Zealand White or Yellow Silver male or female rabbits weighing 3.0–4.0 kg were killed by the intravenous injection of pentobarbital. Both vagi were dissected out, de-sheathed (that is, epineurium removed) and placed in a small compartmentalized Perspex chamber containing physiological salt solution as described previously^{37,38}. Extracellular recordings of the compound action potential were made so that the amplitude of the action potential in both myelinated (α and $\alpha\beta$) and non-myelinated (C) fibres could be recorded separately³⁸. 5-HT caused a dose-dependent reduction in the amplitude of the 'C' fibre action potential at concentrations between 3.5×10^{-7} and 2.5×10^{-6} M without affecting the amplitude of the action potential carried in the faster-conducting α and $\alpha\beta$ fibres. The maximal reduction in the 'C' fibre action potential amplitude caused by 5-HT was $45 \pm 0.4\%$ ($n = 35$), indicating that a subpopulation of 'C' fibres is preferentially affected by 5-HT. Based on these results, 45% reduction in amplitude was considered the 'maximal' (100%) effect. The relative potency of the methylated analogues was measured by running a full cumulative concentration-response curve for 5-HT first and then washing out 5-HT for as long as required for the amplitude of the 'C' fibre action potential to return to its original value (usually 30–40 min). The contact time for each concentration of agonist was 15 min. Methylated analogues were tested subsequently in the same way. Their potencies relative to 5-HT at 50% of the maximum 5-HT effect were then assessed from these dose-response curves for each experiment. Each analogue was tested for antagonistic action of the 5-HT effect by combining the highest concentration which produced no agonist action on its own with 1.5×10^{-6} M 5-HT. None of the analogues had antagonist action when tested in this way. To demonstrate that the reduction in amplitude of the 'C' fibre action potential caused by each of these analogues really resulted from a stimulation of 5-HT M-receptors and not from some other mechanism, the ability of a selective 5-HT M-receptor antagonist to block a maximally effective concentration of each analogue was tested, as such antagonists became available. The agonist action of all analogues was blocked by prior incubation of the nerve with ICS 205-930 (10^{-8} M) for 30 min ($n = 4$). Isolated rat uterus. To study the action of these analogues on D-receptors, isolated uterine strips from oestrogen-treated rats were used as previously described³⁹. Full concentration-response curves for the contractile action of 5-HT were constructed in each case (3×10^{-9} – 3×10^{-6} M). Uteri that did not respond to 10^{-8} M 5-HT were discarded. When the concentration of 5-HT producing a maximal contraction had been established, it was washed out and the methylated analogue tested in the same way. The relative potencies of the methylated analogues were again estimated from the dose-response curves at 50% of the maximal 5-HT effect. As in the vagus nerve, each analogue was tested for antagonism of the 5-HT effect by combining the highest concentration which produced no agonist action on its own with 10^{-6} M 5-HT. None of the analogues had antagonist action when tested in this way. The agonist action of each of the methylated analogues was abolished by prior incubation of the muscle strips with methysergide (10^{-6} M) for 30 min ($n = 6$).



molecule and thereby create analogues with increased specificity for one of these receptors.

Figure 1 shows that this strategy indeed provided highly selective D- and M-agonists. For instance, 2-methyl-5-HT is 2,000 times less potent than 5-HT as an agonist at D-receptors in the rat uterus and yet still possesses ~50% of the potency relative to 5-HT at M-receptors in the vagus nerve. Conversely, S(+)-α-methyl-5-HT has ~50% of the potency of 5-HT in the uterus, but is ~770 times less potent than 5-HT in the vagus nerve.

These two compounds were tested in the other four assay systems (Table 1). The 2-methyl analogue possesses ~50% of the potency of 5-HT on two other M-receptor systems, the rabbit heart and guinea pig ileum. There was no statistically significant difference between the relative potencies of this compound on the three M-receptor systems, indicating that these receptors may be identical. However, the data obtained with S(+)-α-

methyl-5-HT suggests otherwise. Although this compound showed greatly reduced potency relative to 5-HT on all three M-receptor assays, there were differences between the values obtained in each system. Thus, S(+)-α-methyl-5-HT was 770 times less potent than 5-HT on the vagus, 22 times less potent on the heart and only ~11 times less potent on the ileum. Subsequent work with competitive antagonists, as indicated below, confirmed that there are indeed different subtypes of M-receptors in these three tissues.

Radioligand binding data show that the affinity of S(+)-α-methyl-5-HT and 2-methyl-5-HT to 5-HT₁ receptors was ~34 and 370 times, respectively, less than that of 5-HT itself, indicating that these receptors are different from both the D- and M-types (Table 1 and ref. 19). S(+)-α-methyl-5-HT had an affinity similar to that of 5-HT in both the uterus and the competition binding studies using [¹²⁵I]LSD in rat cerebral cortex membranes, whereas 2-methyl-5-HT was devoid of

Table 1 Agonist potency of methylated 5-HT analogues relative to 5-HT (=100)

	Vagus nerve	M-receptors Heart	Ileum	D-receptors Uterus	Cortex-binding sites	
					5-HT ₁	5-HT ₂
5-HT	100	100	100	100	100	100
S(+)-α-methyl-5-HT	0.13 (0.10–0.16)	4.6 (2.8–7.4)	8.9 (5.3–14.9)	49.1 (35.5–67.9)	2.9 (2.5–3.2)	83.5 (56.9–122)
2-Methyl-5-HT	47.3 (41.1–54.5)	52.6 (42.4–65.3)	44.8 (31.8–63.1)	0.05 (0.01–0.07)	0.27 (0.24–0.29)	3.5 (1.5–7.9)

Values are geometric means with 90% confidence limits obtained from a minimum of three independent assays. Methods for estimation of the relative potencies of analogues on the rabbit vagus nerve and rat uterus are given in Fig. 1 legend. Methods for measuring affinities to neuronal 5-HT receptors in the rabbit heart, guinea pig ileum and rat cortex membranes have been published previously^{19,20,34}.

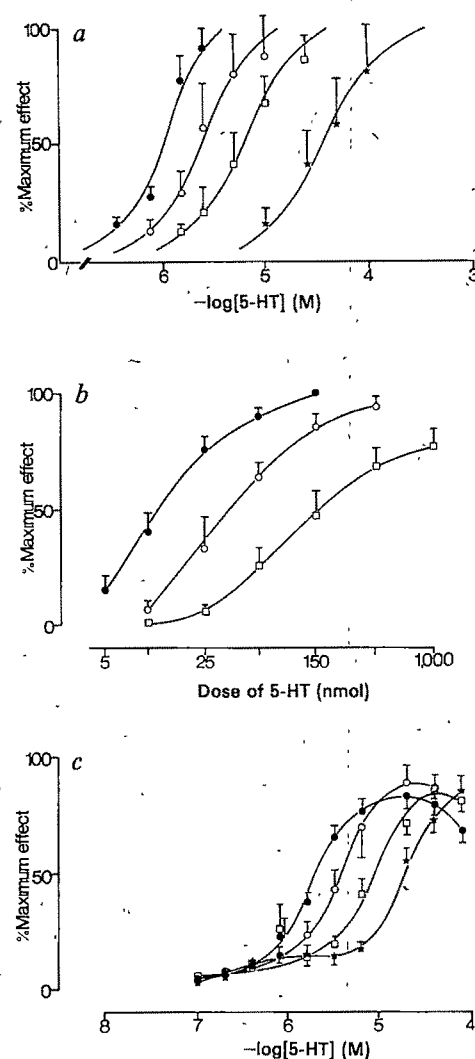
Fig. 2 5-HT concentration-response curves in the presence and absence of competitive M-receptor antagonists. *a*, Rabbit vagus nerve; *b*, rabbit heart; *c*, guinea pig ileum. *a*, Ability of 5-HT to cause a reduction in the amplitude of the 'C' fibre action potential in the absence of antagonist (●) and after prior equilibration of the nerve with compound I (see Table 2) at concentrations of 2×10^{-13} M (○), 10^{-12} M (□) and 5×10^{-12} M (*); $n=4$ in each case. In a separate series of experiments, it was shown that, unlike compound I, the 5-HT D-receptor antagonist methysergide (10^{-5} M) did not influence the 5-HT concentration-response curve in this tissue ($n=3$, data not shown). *b*, Positive chronotropic effect of 5-HT in the absence of antagonist (●; $n=9$) and after prior equilibration of the heart with compound II (see Table 2) at concentrations of 3×10^{-10} M (○; $n=5$) and 10^{-9} M (□; $n=7$). Unlike compound II, methysergide (10^{-5} M) had no effect on the 5-HT concentration-response curve in this tissue ($n=3$, data not shown). *c*, Contractile action of 5-HT in the absence (●) and presence of compound III (see Table 2) at concentrations of 10^{-8} M (○), 3×10^{-8} M (□) and 10^{-7} M (*); $n=4$ in each case. Vertical bars indicate the s.e.m. in each case.

Methods. *a*, In each tissue a cumulative concentration-response curve to 5-HT was established initially, as described in Fig. 1 legend. After washing out the agonist, the nerve was equilibrated with a set concentration of compound I for 60 min and the concentration-response curve to 5-HT was then repeated in the continued presence of the same concentration of compound I. When a concentration of 5-HT sufficient to produce a maximum effect had been applied, the antagonist combination was washed out, the nerve equilibrated for 60 min with a higher concentration of antagonist and the procedure repeated. *b*, Isolated rabbit hearts were perfused by the Langendorff technique and concentration-response curves were constructed for 5-HT in the absence and presence of compound II as described previously⁹. The antagonist was allowed to equilibrate with the tissue for 30 min before repeating the 5-HT concentration-response curves in the continued presence of the same concentration of antagonist. *c*, Concentration-response curves for the contractile action of 5-HT in the presence and absence of compound III were conducted in the guinea pig ileum incubated with 10^{-6} M methysergide as described previously¹⁰. The antagonist was permitted to equilibrate with the ileum for 30 min before repeating the concentration-response curves in the continued presence of the same concentration of antagonist.

relevant effects in either system. Thus, 5-HT₂ receptors in rat cortex are different from M-receptors found on peripheral neurones, although they may be similar to D-receptors found in the uterus and other smooth muscle preparations²¹.

M-receptor antagonists

Our results show that 2-methyl-5-HT and *S*(+)- α -methyl-5-HT are selective M- and D-receptor agonists, respectively. We next attempted to synthesize analogues of 5-HT that retained their affinity and selectivity for M-receptors but that lacked intrinsic activity; in other words, compounds that could occupy these receptors without activating them. Such compounds should behave as competitive antagonists of 5-HT at M- but not D-receptors. By extending the ethylamine chain of 5-HT and making rigid analogues where the terminal nitrogen was included in a tropine or homotropine ring system (see Table 2), we obtained a large series of competitive M-receptor antagonists, with remarkably high affinity and selectivity. Such compounds behave as true competitive 5-HT antagonists on all three M-receptor systems (Fig. 2; Table 2). However, the pA_2 values obtained for compound I (3- α -homotropanyl)-1-methyl-5-fluoroindole-3-carboxylic acid ester or those for compound II (*N*-desmethyl-3- α -homotropanyl)-1-*H*-indole-3-carboxylic acid ester) differed significantly from tissue to tissue ($P < 0.01$, paired *t*-test), indicating that the M-receptors in the vagus, heart and ileum are not identical. Thus, compound I is a highly selective antagonist for the type of M-receptor found in the vagus nerve, whereas compound II shows selectivity for that found in the heart. Both compounds yielded significantly lower pA_2 values for the M-receptors in the guinea pig ileum than for those in



either the vagus or heart, showing the presence of a third type of M-receptor in this tissue. The conclusion that the M-receptors in the ileum and heart differ from one another is in agreement with that of earlier studies where (-)-norcocaine also yielded significantly different pA_2 values in these tissues¹¹. None of the M-antagonists so far synthesized has shown appreciable affinity for uterine D-receptors or for 5-HT₁ or 5-HT₂ receptors in the rat cortex ($pK_D < 5.0$ in all three systems, $n=3$).

As M-antagonists became available, we also tested the selective M-receptor agonist, 2-methyl-5-HT, against them to check that the effects of this agonist in the vagus, heart and ileum were the consequence of M-receptor activation. As shown in Table 2, the pA_2 values obtained were not statistically different ($P > 0.05$, paired *t*-test) from those using 5-HT as agonist, showing that both agonists stimulate a common receptor in each tissue, despite the fact that a different subset of these receptors is found in each of the three tissues, as indicated above. Thus, both 5-HT and 2-methyl-5-HT have full agonist action at each of the three M-receptor subtypes.

The third antagonist listed in Table 2, (3 α -tropanyl)-1-*H*-indole-3-carboxylic acid ester, termed ICS 205-930, is a compound with similar affinity for both the M-receptors in the vagus nerve and for those in the heart, but again shows lower affinity on the ileum. This compound has negligible affinity for uterine D-receptors or for 5-HT₁ or 5-HT₂ receptors in the rat cortex. In a series of ligand binding assays it was also demonstrated that this compound lacks affinity ($pK_D < 6.0$) for α_1 , α_2 , β_1 or β_2 adrenoreceptors, D-1 or D-2 dopamine receptors, H₁ histamine receptors, muscarinic receptors or benzodiazepine receptors (data not shown). This makes ICS 205-930 a potent and selective 5-HT M-receptor antagonist.

Actions of M-receptor antagonists

A bolus injection of 5-HT into the jugular vein of anaesthetized rats induces a vagally mediated reflex bradycardia²². This effect, the von Bezold-Jarisch reflex, can be elicited in several species by various compounds which stimulate sensory nerve endings located in the wall of the right ventricle²³. Recent studies have shown that both metoclopramide and several cocaine analogues can block the 5-HT-induced von Bezold-Jarisch reflex because, in addition to their various other pharmacological properties, they act as neuronal 5-HT receptor antagonists^{24,25}. Consistent with the mediation of this 5-HT effect being through M-receptor activation is the finding that the reflex can also be elicited by the selective M-receptor agonist 2-methyl-5-HT at doses as low as 5 µg per kg (see Fig. 3a), but not by the selective D-receptor agonist *S*(+)- α -methyl-5-HT at doses as high as 1,000 µg kg⁻¹ (*n* = 5), and that the ability of 5-HT to induce the von Bezold-Jarisch reflex is not prevented by pretreatment of the rats with the D-receptor antagonist methysergide (Fig. 3b). In contrast, an intravenous dose of 1 µg per kg of the selective M-receptor antagonist ICS 205-930 caused a parallel rightward shift of the 5-HT dose-response curve, such that a fourfold increase in the dose of 5-HT was necessary to produce the same reflex reduction in heart rate as occurred before administration of the antagonist (Fig. 3b). Thus, M-receptors are responsible for the initiation of this reflex by 5-HT and can be blocked by very low doses of ICS 205-930.

Inhibition of 5-HT-induced pain

5-HT causes pain when applied to a blister base on the human forearm and it markedly potentiates the vascular pain produced by bradykinin^{26,27}. Therefore, we investigated whether these actions are mediated by D- or M-receptors.

The M-receptor agonist 2-methyl-5-HT was as effective as 5-HT in inducing a pain response, whereas the D-receptor agonist *S*(+)- α -methyl-5-HT was ~100 times less potent (Fig. 4). Consistent with the pain-producing effects of 5-HT being mediated through M-receptor activation was the finding that the D-receptor antagonist methysergide did not inhibit 5-HT-induced pain, whereas ICS 205-930 at the low concentration of 10⁻⁸ M caused a marked rightward shift of the 5-HT pain dose-response curve. The inhibitory effect of ICS 205-930 could be

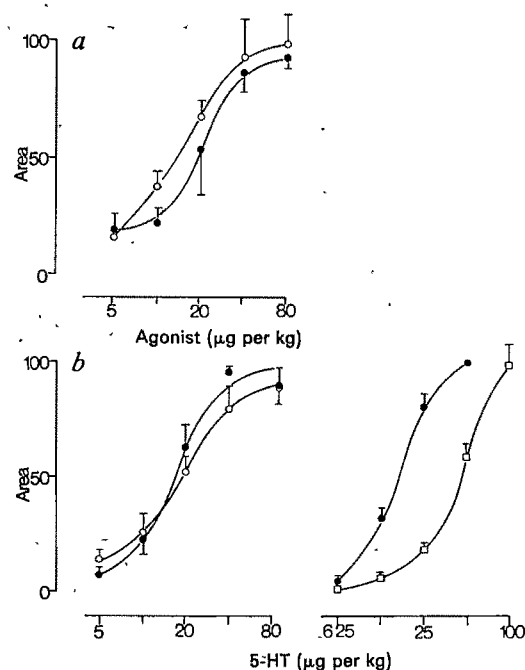


Fig. 3 The von Bezold-Jarisch effect in anaesthetized rats. *a*, Dose-response curves for 5-HT (●; *n* = 5) and 2-methyl-5-HT (○; *n* = 4). The selective D-agonist *S*(+)- α -methyl-5-HT was totally inactive up to a dose of 1,000 µg per kg, the highest one tested (*n* = 5, data not shown). *b*, Dose-response curves to 5-HT before (●; *n* = 5) and 5 min after the intravenous administration of the 5-HT D-receptor antagonist methysergide (○; 100 µg per kg; left panel; *n* = 4) or the M-receptor antagonist ICS 205-930 (□; 1 µg per kg; right panel; *n* = 5). Vertical bars indicate s.e.m. in each case. 'Area' refers to the area under the time-heart rate curve. **Methods.** Male rats weighing 220–270 g were anaesthetized by injecting urethane (1.25 g per kg) into the peritoneal cavity. Blood pressure and heart rate were recorded by standard techniques using a Statham transducer. Injections were made into the jugular vein. Rapid bolus injections of 5-HT (5–100 µg per kg) given at 5-min intervals elicited abrupt dose-related reductions in cardiac rate of short duration and reproducible magnitude. The area under the curve describing the reflex bradycardia (ordinates) quantified the effect.

Table 2 Antagonism of M-receptors in isolated tissues

Antagonist	Tissue	Agonist	pA_2 value $\pm$ s.e.m.	Slope of $\log(DR-1)$ on $\log(B) \pm$ s.e.m.
<chem>CN1[C@H]2CC[C@@H]1C(=O)C3=CC=C(C=C3)C=C4C(=O)N(C)CC4</chem> Compound I	Vagus	5-HT	13.10 $\pm$ 0.78	0.76 $\pm$ 0.21
	Vagus	2-Methyl-5-HT	13.55 $\pm$ 0.77	0.70 $\pm$ 0.19
	Heart	5-HT	10.11 $\pm$ 0.09	1.17 $\pm$ 0.29
	Ileum	5-HT	9.10 $\pm$ 0.13	0.91 $\pm$ 0.15
<chem>CN1[C@H]2CC[C@@H]1C(=O)C3=CC=C(C=C3)C=C4C(=O)N(C)CC4</chem> Compound II	Vagus	5-HT	8.90 $\pm$ 0.18	0.82 $\pm$ 0.12
	Heart	5-HT	9.83 $\pm$ 0.14	1.26 $\pm$ 0.27
	Heart	2-Methyl-5-HT	9.70 $\pm$ 0.05	1.16 $\pm$ 0.24
	Ileum	5-HT	7.74 $\pm$ 0.22	0.98 $\pm$ 0.17
<chem>CN1[C@H]2CC[C@@H]1C(=O)C3=CC=C(C=C3)C=C4C(=O)N(C)CC4</chem> Compound III (ICS 205-930)	Vagus	5-HT	10.23 $\pm$ 0.19	0.95 $\pm$ 0.18
	Heart	5-HT	10.63 $\pm$ 0.22	0.92 $\pm$ 0.46
	Heart	5-HT	7.85 $\pm$ 0.10	1.22 $\pm$ 0.19
	Ileum	2-Methyl-5-HT	8.03 $\pm$ 0.05	0.93 $\pm$ 0.03

Experiments to assess the affinities of three competitive M-receptor antagonists against 5-HT or 2-methyl-5-HT on the three isolated tissues were performed as described in Fig. 2 legend. The pA_2 and slope values were obtained by transforming the data from concentration-response curves to 5-HT or 2-methyl-5-HT conducted in the absence or presence of the respective antagonist from a minimum of three independent experiments, as described elsewhere³⁵. DR , dose ratio (the factor by which the agonist concentration has to be increased in the presence of the antagonist to obtain a response identical in magnitude to that recorded in the absence of the antagonist); B , antagonist concentration. If 5-HT and the antagonist under consideration compete for a common site, then $\log(DR-1) = \log(B) - \log K_B$, where K_B is the apparent dissociation constant for the antagonist-receptor interaction³⁶. In this case the regression of $\log(DR-1)$ on $\log(B)$ (Schild plot³⁵) yields a straight line of unity slope and a pA_2 value of $-\log K_B$. Slope and pA_2 values given here were obtained from linear regression analysis of the Schild plots. In all three tissues, compounds I, II and III behaved as true competitive antagonists, causing parallel rightward displacements of the agonist concentration-response curves without significantly reducing the maximum effect. In addition, the slope of $\log(DR-1)$ on $\log(B)$ was not significantly different from unity (*t*-test), showing that 5-HT and the antagonists compete for common receptor sites in these tissues.

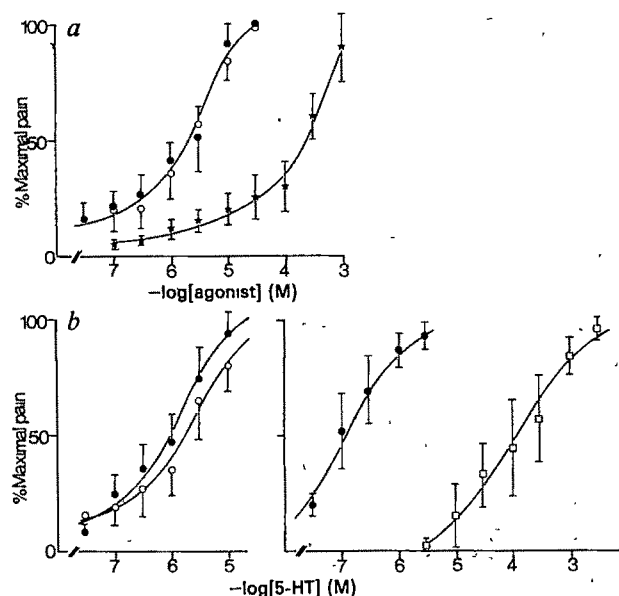


Fig. 4 Painful effects of 5-HT and methylated analogues when applied to a blister base on the human forearm. *a*, Effects of 5-HT (●; $n=5$), 2-methyl-5-HT (○; $n=3$) and *S*(+)- α -methyl-5-HT (*; $n=3$) in causing pain. *b*, Concentration-response curves for 5-HT in the absence (●; $n=4$) or presence of 10^{-5} M methysergide (○; $n=3$; left panel) or 10^{-8} M ICS 205-930 (□; $n=4$; right panel). Antagonists were applied to the blister base for 20 min before applying 5-HT in their continued presence. Vertical bars indicate s.e.m. in each case.

Methods. Double-blind studies were conducted on five healthy male volunteers aged 32–55 yr. Blisters were produced on the medial aspect of the forearm as described previously²⁶. Increasing concentrations of 5-HT (10^{-8} – 10^{-5} M) were applied to the blister base in Krebs–Ringer solution. The contact time in each case was 2 min. Subjects were asked to register the pain experienced during each 2-min interval by moving a lever graduated on a 0–3 rating scale (0=no pain, 1=slight pain, 2=moderate pain, 3=severe pain) coupled to a linear integrator that then recorded the area under the 'pain intensity-time curve'. The concentration of 5-HT was increased every 2 min until one was reached where no further pain was produced. In this way, a cumulative dose-response curve for 5-HT could be constructed.

fully reversed on washing off the antagonist with buffer for 60 min.

To confirm that this effect of ICS 205-930 reflected a specific antagonism of 5-HT and not a general time-dependent reduction in the sensitivity of the blister base to all painful stimuli, dose-response curves for bradykinin (10^{-8} – 10^{-6} g ml⁻¹) were performed before, during and after incubation of the blister with ICS 205-930. The three dose-response curves were virtually superimposed and the maximum response similar at each time point (data not shown). Thus, in contrast to its effect on 5-HT responses, ICS 205-930 did not inhibit bradykinin-induced pain.

Concomitant with the pain response, 5-HT caused a wheal and flare reaction that presumably occurred as a result of the activation of sensory nerve endings in the skin and the consequent liberation of substance P by an axonal reflex mechanism²⁸. This was also inhibited by 10^{-8} M ICS 205-930. At the higher concentrations of 5-HT needed to induce pain in the presence of the antagonist, the wheal and flare response reappeared.

5-HT also caused a marked potentiation of bradykinin pain responses and this could be abolished by ICS 205-930 (Fig. 5). This inhibitory effect was partly reversed on washing off the antagonist for 20 min. Thus, ICS 205-930 not only inhibits pain responses to 5-HT in this system, but also blocks its ability to potentiate bradykinin responses.

These studies show that 5-HT induces its painful effect in humans through activation of neuronal M-receptors and that this can be selectively and reversibly inhibited by the M-receptor antagonist ICS 205-930.

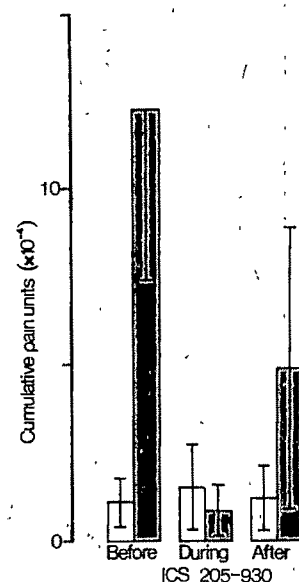


Fig. 5 Potentiation of the painful effects of bradykinin on the human blister base by 5-HT and inhibition of this effect by ICS 205-930. Pain produced by a solution containing 10^{-8} g ml⁻¹ bradykinin alone (open columns) or in combination with 10^{-4} M 5-HT (hatched columns) was recorded before, during and after incubation of the blister base with 10^{-8} M ICS 205-930. Mean values obtained from five male volunteers are given. Vertical lines on each column indicate s.e.m.

Methods. Double-blind studies were performed in a further group of male volunteers aged 22–45 yr as described in Fig. 4 legend. In this series of experiments 10^{-8} g ml⁻¹ bradykinin was applied to the blister base first and the response recorded. This concentration of bradykinin produced a small pain response ('before', open column). It was subsequently washed off and the blister base incubated with buffer for 20 min before 10^{-4} M 5-HT was applied repeatedly. Initially this induced a large pain response as expected, but this subsided over the next 2–6 min despite repeated reapplication of the same 5-HT concentration. Subsequently 10^{-8} g ml⁻¹ bradykinin was applied to the blister in the continued presence of 10^{-4} M 5-HT for 2 min, resulting in a 10-fold increase in the bradykinin response ('before', hatched column). The bradykinin/5-HT combination was then washed off for 20 min with buffer, after which the blister base was incubated with 10^{-8} M ICS 205-930 for a further 20-min period. The responses to bradykinin (10^{-8} g ml⁻¹; 'during', open column) and the bradykinin/5-HT combination (10^{-8} g ml⁻¹ and 10^{-4} M, respectively; 'during', hatched column) were then recorded in the continued presence of 10^{-8} M ICS 205-930. ICS 205-930 completely inhibited the potentiation of bradykinin pain caused by 5-HT. This inhibitory effect of ICS 205-930 was partly reversed on washing off the antagonist for 20 min ('after').

Discussion

Compounds such as the ergot alkaloids, which possess the ability to inhibit the action of 5-HT at D-receptors on smooth muscle cells or at postsynaptic 5-HT₂ receptors in the central nervous system, have been available for many years²⁹. Here we describe the synthesis of highly potent and selective antagonists of another class of 5-HT receptors, M-receptors, located on peripheral nerves. Significant differences in the affinity constants obtained with the same competitive antagonist on different bioassay systems indicate that there are at least three distinct subclasses of these M-receptors.

During the course of this work we became aware of a similar project by J. Fozard and his colleagues. Based on earlier observations that (–) cocaine and structurally related compounds act as competitive antagonists at neuronal 5-HT receptors in the rabbit heart and guinea pig ileum^{8,10,11}, this group synthesized analogues with improved potency and selectivity, which yielded a series of compounds that are antagonists at 5-HT M-receptors in the heart³⁰. A report describing the pharmacological properties of one of these drugs, MDL 72222 (3,5-dichloroben-

zoilytropine ester), appeared during the preparation of this manuscript³¹. Unlike the indoletropanyl and homotropanyl esters synthesized by our group, none of these benzoyltropine analogues blocks 5-HT M-receptors in the guinea pig ileum or exhibits the remarkably high potency for vagal M-receptors achieved with many of our antagonists¹⁸. Indeed, some of the indole compounds we have synthesized are the most potent drugs of any pharmacological class so far described.

Studies in humans and rats with the selective antagonist ICS 205-930 show that M-receptors are present on sensory nerve endings in the skin and right ventricle of the heart. The ability

of M-receptor antagonists to block selectively the painful effects of 5-HT when applied to a blister base on the human forearm suggests that such compounds might present a novel approach to the management of conditions such as migraine and cluster headaches, where 5-HT may be involved in the genesis of vascular pain^{27,32}. This possibility has been discussed previously in detail²⁴ and encouraging preliminary clinical results with MDL 72222 support this contention³³.

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LETTERS TO NATURE

Statistical significance of the 59-s periodicity of Geminga and 1EO630+178

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Bignami *et al.*¹ have reported evidence for an increasing 59-s period in the X-ray emission from the high-energy ($E \geq 50$ MeV) γ -ray source Geminga. Their claim is based on results of a search for periodicities at around 59s in the emission from the X-ray source 1EO630+178, which has been proposed as a possible counterpart of Geminga². We show here that the primitive indication by SAS-2 (ref. 3) of a 59-s periodicity in the γ -ray flux from Geminga is not statistically significant, and because it has also not been confirmed by COS-B (ref. 4), must be disregarded; the statistical significance of the results obtained from periodicity searches in the X-ray data is significantly lower than that reported by Bignami *et al.*¹. As a consequence, the identification of 1EO630+178 with the γ -ray source 2CG195+04 (Geminga) through a 59-s pulsation cannot be claimed.

The first suggestion of a possible 59-s periodicity in the Geminga γ -ray emission was derived from SAS-2 data³. The analysis was performed on a data set consisting of 121 photons, energy >35 MeV, detected over three separate observation intervals, each about 1 week long, with 37, 28 and 56 counts respectively. These data were searched for pulsation and the result was a 25-bin phase histogram with a χ^2 of 78 for a trial period of 59.0074s, increasing at a constant rate of 2.23×10^{-9} s per s. Without entering into the details of the analysis method used by Thompson *et al.*³, we can evaluate the chance occurrence probability of that result. For 121 counts uniformly spread over 25 bins the central limit theorem does not hold and the theoreti-

cal probability distribution of the χ^2 cannot be used. We derived the actual probability distribution by making a Monte-Carlo simulation of 10^6 sets of 121 counts uniformly distributed in three observation intervals as in the SAS-2 experimental conditions. The result is shown in Fig. 1. It is evident that $P(\chi^2 \geq 78) \approx 1 \times 10^{-6}$. Therefore, no more than 1,000 trials are allowed in order for the SAS-2 indication to be statistically significant. The number of trials used by the SAS-2 team was actually 8×10^4 around an input period of 58s (D. Thompson, personal communication), which implies a global chance occurrence probability of $\sim 8\%$ for the reported effect. Furthermore, as the choice of the input period was not derived from independent studies but from an investigation of the same γ -ray data, the implicit number of trials made to identify it should also be taken into account. Therefore, the value of 8% is a lower limit. On the other hand, Thompson *et al.*³ were aware of the weakness of their indication. After showing the effect they, in fact, declared that "... in view of the number of trials used ... the evidence for this periodicity is not statistically compelling but is worth examination by future experiments".

COS-B has observed the general direction of Geminga five times. Bignami *et al.*¹ refer to the results of a preliminary analysis of the first observation, made from 17 August to 17 September 1975, during which COS-B was pointing at the Crab pulsar and Geminga was visible at 15° off axis⁵. The quoted Caravane Collaboration paper stated a confirmation (1% chance occurrence probability) of the 59-s effect claimed by SAS-2. However, the χ^2 of the histogram, obtained after 133 trials was 43.6 and then, in our reevaluation, the global chance occurrence probability of the COS-B effect is $1.2 \times 10^{-3} \times 133 \approx 16\%$. Later, the COS-B team extended the analysis when the data from two more observations became available. Due to an increase in source counts by a factor of ~ 5 and more optimized selections, a much better signal-to-noise ratio than in the previous analysis became available. As a result, Masnou *et al.*⁴ concluded that "... no periodicity is observed around the 59s value". Note that the γ -ray flux of Geminga has remained constant within the statistical uncertainties from the SAS-2 (ref. 3) observations to those of COS-B (ref. 6). Therefore, applying the reasonable

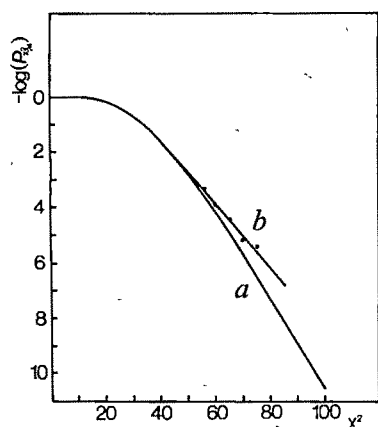


Fig. 1 *a*, Theoretical probability distribution of the χ^2 with 24 d.f. *b*, Probability distribution of the variable $y = \sum(x_i - \bar{x})^2 / \bar{x}$ where $\bar{x} = 121/25$ and x_i is the content of the i th bin of the histogram obtained by folding, with a period of 59s, the arrival times of 121 events, uniformly distributed in three time intervals of 7, 5 and 7 days, respectively (total elapsed time between the beginning of the first week and the end of the third week is 131 days, as in the SAS-2 case).

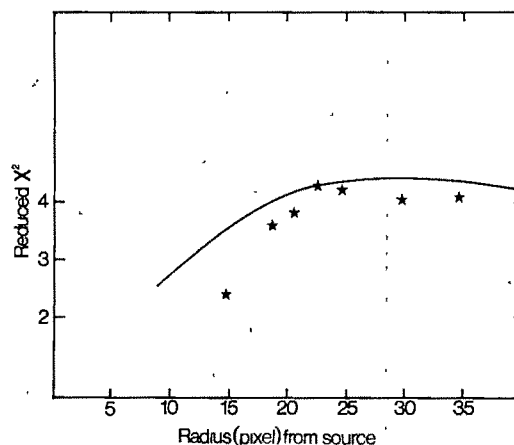


Fig. 2 Distribution of the expected reduced χ^2 as a function of radius R (solid line) compared with reduced χ^2 values (*) obtained in ref. 1 by folding the IPC 10371 data with a period of 59.737s.

hypothesis that the shape of the light curve and the pulsed fraction of the flux has also remained stable, we expect a strong signal in the COS-B data due to the high counting statistics. In fact, the peak of the SAS-2 light curve when scaled to the COS-B exposure of Geminga would show up at the level of at least 16σ in the case of observation period 14 alone. Despite all the effort devoted by the Caravane Collaboration to detect periodicity effects in the Geminga data, no such result has been found.

The absence of a timing signature in the γ -ray emission from Geminga makes it impossible to identify a counterpart at other wavelengths. This also applies to the candidate-counterpart X-ray source 1E0630+178. Nevertheless, Bignami *et al.*¹ have centred the temporal analysis of the X-ray data of 1E0630+178 at the disclaimed Geminga period of 59s. The reported X-ray time analysis can also be criticized for several reasons:

(1) The chance occurrence probability of the 59-s effect in the Einstein 10371 data has been incorrectly calculated. As a result of the 20 trial periods scanned, a reduced χ^2 of 3.94 was obtained, corresponding to a global chance occurrence probability of $\sim 5 \times 10^{-5} \times 20 = 1 \times 10^{-3}$. Bignami *et al.*¹ quote a probability of 2×10^{-5} for the same data. This error is due to two reasons. First the single trial probability used to obtain a reduced χ^2 of 4.26 (9 d.f.) was 1.5×10^{-5} (ref. 7) and not 1×10^{-6} . Second, the value of 4.26, used instead of 3.94, is the result of fine scanning performed in addition to the initial 20 trials. Therefore, the appropriate number of periods searched is higher than 20, the value used by Bignami *et al.*¹. The same criticism applies to the combined data of IPC 10371 and HRI 8353, again an error in the single trial probability and the underestimated number of trials.

(2) Bignami *et al.*¹ fixed, *a priori*, the radius R (in pixels of 8 arc s) from the source position at $R = 23$ in their event selection and, correctly, discuss, *a posteriori*, whether the distribution of χ^2 values as a function of R behave as expected for a genuine source effect. This can be better judged from Fig. 2 which shows the reduced χ^2 expected for various values of R as derived from the relation $\text{reduced } \chi^2 = C \times NS^2 / NT + 1$ (ref. 8) (where NS is the number of source counts and NT is the total number of counts taken from Fig. 1 of ref. 1 and C is obtained by normalization at $R = 23$). Figure 2 shows that the χ^2 value at $R = 23$ is an upward fluctuation from the expected general trend. Therefore, the probability level of 1×10^{-3} is the lower limit to the probability that the reported effect is due to a random fluctuation.

(3) The useful observation time of the first Einstein observation (IPC 3333) is only 0.29 of that of the second and we expect a reduced χ^2 of 2.1 (chance occurrence probability of 50% in 20 trials). The measured value of 3.6 can only be reconciled with the IPC 10371 result and the SAS-2 indication, on which this search has been based, if three parameters are adjusted, namely the $\dot{P}$ value, the intensity of the pulsed emission from 1E0630+178, and possibly the shape of its light curve. Therefore, the effect found in the IPC 3333 data cannot be considered as independent evidence of a genuine effect, nor can the significances derived from the two observations be combined without taking into account the additional degrees of freedom.

Finally, we dispute the arguments based on the Zyskin and Mukanov results⁹ derived from measurements at ultra-high energies in 1979 and 1981. First, the result derived from the 1979 observations is declared by the authors to be statistically weak (6% chance occurrence probability). Second, for the 1981 data Zyskin and Mukanov show their 'light curve' with 19 bins and a χ^2 of 43.6. To obtain this effect as a random fluctuation, they use 10 d.f. in their probability calculation instead of 18. Using 18 d.f. the probability increases up to $6 \times 10^{-4} \times 100 = 6 \times 10^{-2}$ (compared with their quoted probability of 5×10^{-4}). Therefore, the result of the analysis of the 1981 data cannot be considered significant either. In addition, the obtained period is not consistent with the P and $\dot{P}$ derived from the Einstein or SAS-2 data and cannot be taken as independent evidence.

We conclude that the identification of Geminga with 1E0630+178 and the consequent speculations made by Bignami *et al.* are based on arguments too weak to be conclusive. The mystery of 2CG195+04 does not seem to be solved, although 1E0630+178 continues to be an interesting candidate counterpart.

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Joint STARE and SABRE radar auroral observations of the high-latitude ionospheric convection pattern

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The projection of the magnetospheric convection electric field into the ionosphere drives an asymmetric two-cell pattern of plasma circulation in the auroral zone. The Scandinavian Twin Auroral Radar Experiment (STARE) and the Sweden And Britain auroral Radar Experiment (SABRE) are two similar and independent coherent radar systems which can estimate *E*-region electron drift velocities at auroral latitudes. By combining their measurements, we have monitored simultaneously, for the first time, the convection flow pattern over 8 degrees of latitude. We show here how this allows a more extensive study of the nightside (Harang discontinuity) and dayside (cusp) boundaries between the cells, and enables spatial and temporal features of the flow pattern to be resolved.

The STARE and SABRE systems consist of two multi-beam radars that are sensitive to electrostatic ion-acoustic waves, which are produced in association with current flows in the auroral zone E-layer. The phase velocity of these irregularities is approximately equal to the electron drift velocity¹, and can be determined from the mean Doppler shift of the transmitted radar signal. Each radar measures the backscatter intensity and Doppler velocity of irregularities at each of 50 ranges within eight beams, providing a total common viewing area in the auroral E-region of ~200,000 km². The spatial resolution is ~20 × 20 km and the temporal resolution is usually 20 s. The Doppler shifts measured by the two radars are combined to give the electron drift velocity in a two-dimensional array of geographical locations within the viewing area. Recent EISCAT/STARE comparisons² indicate that the coherent auroral radar underestimates the higher flow speeds, although the directions are accurate. An empirical correction factor has been developed, the validity of which is yet to be established for SABRE. No correction has therefore been applied to the data presented here.

The STARE system³ has been operated by the Max-Planck-Institut für Aeronomie (MPI), Lindau, FRG, since 1977 and consists of radars at Malvik in Norway and Hankasalmi in Finland. SABRE⁴ was completed in 1982 and is operated jointly by Leicester University, UK and by MPI. The two SABRE radars are located at Wick in Scotland, and at Uppsala in Sweden.

In 1961, Axford and Hines⁵ proposed that viscous interaction between the solar wind and a closed magnetosphere would produce a high-latitude two-cell ionospheric convection pattern. At the same time, Dungey⁶ introduced the concept of solar wind interaction with an open magnetosphere through magnetic reconnection. This mechanism involves the merging of geomagnetic field lines on the dayside of the Earth's magnetopause with the interplanetary magnetic field (IMF). As the IMF is frozen into the solar wind, the opened field lines are 'dragged' across the poles, transporting plasma in an anti-sunward direction. The field lines reconnect in the magnetospheric tail and the return flow is on closed field lines around the flanks of the magnetosphere. It is generally accepted that both viscous interaction and magnetic reconnection processes drive plasma convection within the magnetosphere and that the ionospheric projection of these motions takes the form of a two-cell pattern (Fig. 1). Although the gross features of this pattern have now been well established, recent observations^{7,8} indicate that the shape and relative sizes of the convection cells are considerably modified by geomagnetic substorms and by the orientation of

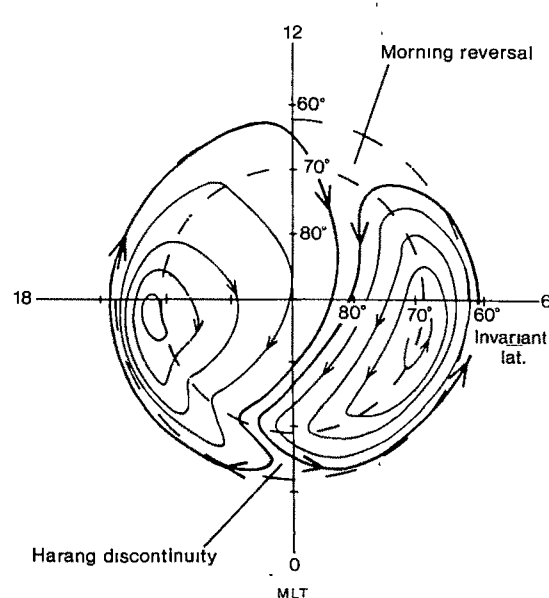


Fig. 1 The two-cell convection pattern, plotted in invariant latitude against magnetic local time (MLT), for moderately disturbed geomagnetic conditions. Superimposed is the path taken by the combined STARE and SABRE viewing area, as the Earth rotates beneath the cell pattern.

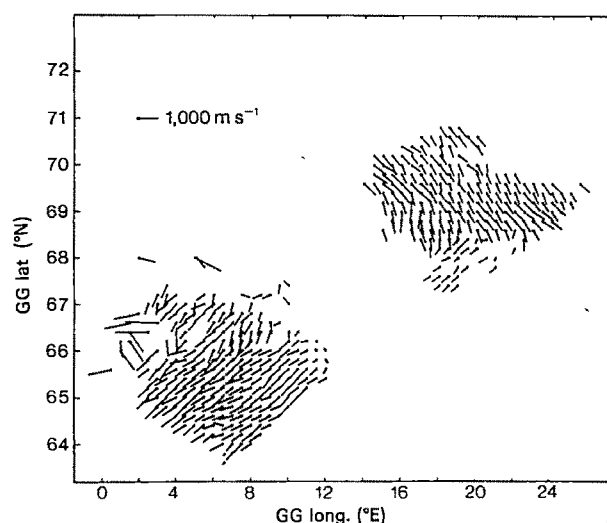


Fig. 2 Simultaneous STARE and SABRE flow velocity observations made at 1111 UT 6 September 1982 in the Harang discontinuity region. The velocities are given as a function of latitude and longitude.

the IMF. From several days of STARE observations, it has been suggested⁹ that as magnetic activity increases: (1) the convection flow speeds increase; (2) the flow pattern expands; (3) the morning cell is enlarged with respect to the evening cell; and (4) the whole convection pattern rotates clockwise (in the Northern Hemisphere) towards earlier local times.

We now consider simultaneous STARE and SABRE observations of flows associated with both the morning and the evening convection reversals. An example of the convection flows measured in the vicinity of the evening reversal or Harang discontinuity is shown in Fig. 2 which indicates the convection flow vectors at 1111 UT 6 September 1982 in both viewing areas, with the SABRE field of view located to the south-west. The length and direction of each vector represent the flow speed and direction respectively. The flow direction in the STARE field of view is predominantly eastward, while that in the SABRE viewing area is westward. From an examination of the temporal development of the flow pattern, it was established that at this

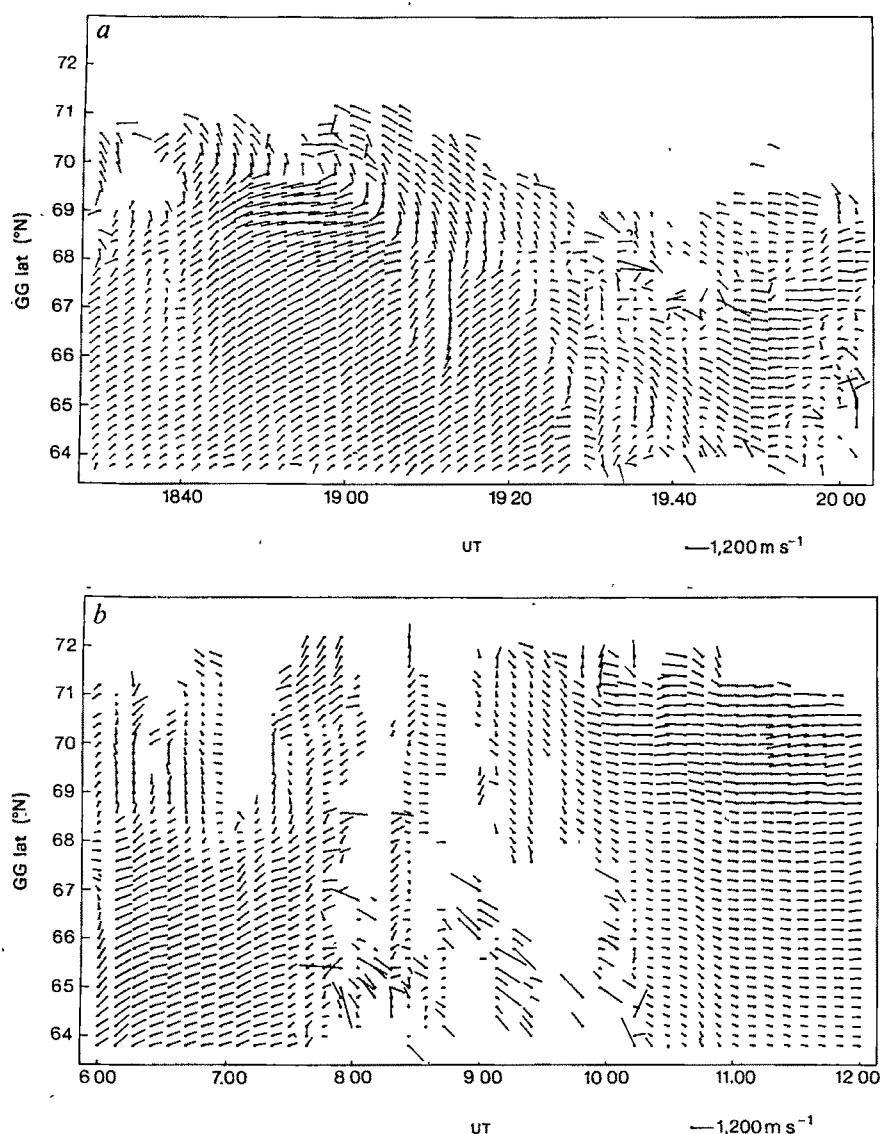


Fig. 3 Combined STARE and SABRE convective flow velocity measurements for *a*, 6 September 1982, 1830–2000 UT; *b*, 7 August 1982, 0600–1200 UT. The velocities are given as a function of latitude and time, averaged over the longitude range 8–18° E.

time the viewing areas lie either side of the Harang discontinuity, with STARE and SABRE observing the morning and evening convection cells respectively. Figure 3*a* illustrates the convection flow velocities, as a function of latitude and time, averaged over the longitudinal interval 8–18° E. This is chosen to include both the eastern edge of the SABRE viewing area and the western edge of the STARE viewing area. Because of the longitudinal displacement between the two viewing areas, difficulties arise with this averaging process when the convection flow is not spatially uniform. The plot covers the period 1830–2000 UT, during which time the Harang discontinuity passed through both viewing areas. Figure 3*a* indicates the transition from the region of westward flow, which is associated with the evening convection cell, to that of eastward flow associated with the morning cell. The discontinuity moves equatorward with time throughout the entire latitude range, although the penetration speed of the eastward flow is latitudinally dependent. The equatorward drift is $\sim 250 \text{ m s}^{-1}$ within the STARE viewing region but has increased to $1,000 \text{ m s}^{-1}$ by the time the discontinuity passes through the SABRE viewing area. The sudden change at around 68° N arises from the longitudinal resolution limitation mentioned above, and indicates the presence of strong spatial velocity variations in the auroral E-region. Owing to the exceptionally high level of magnetic activity at this time ($K_p = 7$), the convection cell pattern has expanded equatorwards, and the strong auroral zone flows normally found at latitudes above 67° N are located within the latitude range covered by SABRE. The STARE radar measures weaker flows characteristic of the

polar cap region. In quiet geomagnetic conditions, the Harang discontinuity would be expected to occur at 2300 UT (refs 7, 9). This particular reversal took place between 1850 and 1940 UT, implying a clockwise rotation of the entire cell pattern by $\sim 3\text{--}4 \text{ h}$. Despite the magnetically-active conditions, the gross features of the observed flows are in good agreement between the two systems, demonstrating that STARE and SABRE may be usefully combined for increased latitudinal coverage in the morphological study of convection flows in the auroral ionosphere.

Although the Harang discontinuity is frequently observed by STARE and SABRE, convection flows associated with the morning reversal are very weak at these latitudes. In highly disturbed conditions, however, strong flows can occur in this region at latitudes below 64° N (refs 8, 10). Figure 3*b* is an example of a morning sector observation, illustrating the temporal development of the plasma flow pattern in the vicinity of the convection reversal, for the time interval 0600–1200 UT 7 August 1982. The value of K_p was +7 during the period 0600–0900 UT and –7 between 0900 and 1200 UT. In contrast to the evening sector example, the change in flow direction is from eastwards to westwards, and the flow pattern displays a throat-like structure which widens with decreasing latitude. Although auroral radar observations of the morning reversal are rare, they are of particular interest because in this local time sector the Earth's field lines map directly into the outer magnetosphere. Measurements made in this region of the high-latitude ionosphere can, therefore, be used to infer the properties of the plasma in the polar cusp, which is the highly dynamic zone of direct interaction

between the Earth and the interplanetary environment. Future studies involving the correlation of STARE and SABRE cusp flow observations with the behaviour of the IMF will enhance our understanding of the field line reconnection mechanism by which the solar wind drives the high-latitude convection cell pattern.

The STARE radar was constructed and is operated by MPI, Lindau, FRG. SABRE was constructed and is operated jointly by MPI and the Department of Physics, Leicester University, UK.

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Increasing abundance of CBrClF₂ in the atmosphere

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The importance of bromine chemistry was recognized shortly after Molina and Rowland¹ had drawn attention to chlorine atom-catalysed destruction of ozone. Bromine atoms are even more efficient catalysts than chlorine^{2,3}, and both may act synergistically to deplete ozone in the lower stratosphere⁴. Some measurements of bromine-containing source gases, such as CH₃Br, CHBr₃, CH₂BrCH₂Br, CH₂Br₂, CBrF₃ and CBrClF₂ have been reported for the troposphere⁵⁻⁸, their vertical distribution in the stratosphere is unknown except for CBrF₃ (CFC-13B1) and CH₃Br (ref. 6). These, together with the fully halogenated CBrClF₂ (CFC-12B1), are probably the most important source gases for stratospheric bromine radicals. We report here the first stratospheric profiles of CBrClF₂, up to 25 km, measured at 44° N during three consecutive years. From these data it follows that the atmospheric abundance of this halocarbon is growing rapidly, at a rate of 20% per year in the Northern Hemisphere, corresponding to a worldwide emission of $\sim 3.6 \times 10^3$ tons per year.

Like CFC-13B1, whose vertical distribution has already been measured⁶, CFC-12B1 has an anthropogenic origin: both substances are used as fire extinguishers. Their ultraviolet absorption characteristics are similar to those of the corresponding chlorine-containing species, but shifted towards longer wavelengths. Thus halogen atoms are released from these molecules in the lower stratosphere, leading to overall atmospheric lifetimes of 29-42 yr and 62-117 yr for CFC-12B1 and CFC-13B1, respectively⁹. Both substances are abundant at ~ 1 p.p.t.v. (parts per 10^{12} by volume)^{5,8,10}. Compared with CH₃Br, the main natural bromine-containing halocarbon whose tropospheric mixing ratios were found to lie between 5 and 20 p.p.t.v. (refs 5, 8, 10), the anthropogenic CFC-12B1 and CFC-13B1 contribute between 10 and 30% to the present organically-bound bromine.

Stratospheric profiles of CBrClF₂ were obtained by analysing air samples collected cryogenically by means of neon-cooled samplers. The new MPAE samplers¹¹ allow the collection of samples of 30 litres STP (standard temperature and pressure) at 15 different altitudes up to 40 km. CBrClF₂, among other halocarbons, was analysed by gas chromatography with an electron capture (EC) detector. A 3 m $\times$ 2 mm internal diameter (i.d.) glass column packed with OV-101 on Chromosorb WHP

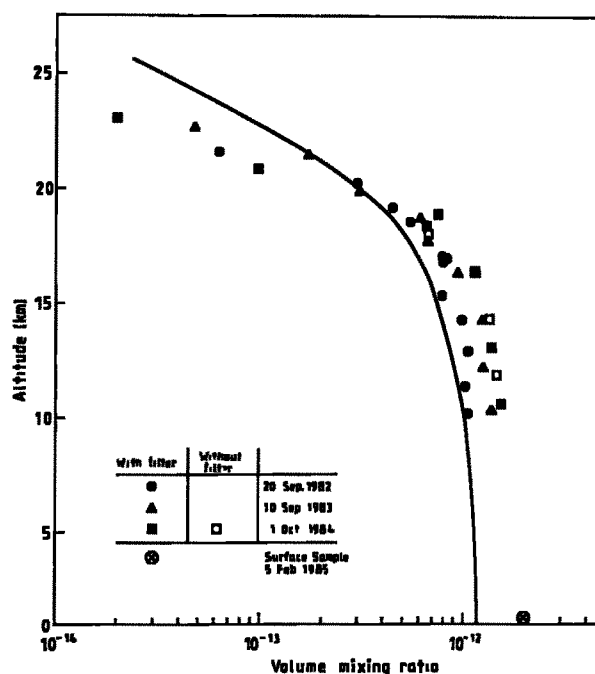


Fig. 1 The vertical distribution of CBrClF₂ (CFC-12B1) measured at 44°N during three consecutive years. The theoretical profile was computed by means of a time-dependent one-dimensional model, for 20 September 1982.

80-100 mesh was used for separation, with temperatures varying between -50 and +60 °C and argon/methane (10%) as the carrier gas¹². In combination with a cryotrap preconcentrator, CFC-12B1 is analysed with a precision of $\pm 5\%$, the detection limit being 0.03 p.p.t.v. The absolute calibration is achieved by two-step static dilution with $\sim \pm 10\%$ accuracy.

Results from three balloon flights carried out over southern France, on 20 September 1982, 10 September 1983, and 1 October 1984, are presented in Fig. 1. Most samples were collected with a copperwool filter attached to the air intake, to destroy incoming ozone¹³. Sampling tests performed in simulated stratospheric conditions, with gas mixtures containing 5 p.p.t.v. of CFC-12B1, gave no indication of sampling errors exceeding the analytical precision ranges of $\pm 5\%$. In contrast to some other halocarbons, CFC-12B1 does not change in the sampler when ozone is added; thus no systematic differences occur between samples collected with or without this filter. All analyses were completed within 2 months after each flight.

By repetitive analyses and cross-checks, it was found that the sensitivity of the analytical system had changed in 1984. Thus, a correction factor of (1.14 ± 0.04) had to be applied to the 1982 and 1983 profiles. No change of sensitivity was found between 1982 and 1983. The step-like structures observed in the profiles shown in Fig. 1 reflect real variations which were also observed in the profiles of other source gases analysed together with CFC-12B1. Such structures were found to be due to large-scale advection processes^{14,15}.

If the lowest two samples of each flight are considered to be representative of high-tropospheric conditions (in fact, the tropopause was between 11 and 12 km for all three flights), the abundance of CFC-12B1 increased from 1.03 p.p.t.v. measured on 20 September 1982, to 1.31 p.p.t.v. on 10 September 1983, and to 1.49 p.p.t.v. on 1 October 1984. These values correspond to relative growth rates of 28% per year for 1983 and 12% per year for 1984, or 20% per year averaged over both years. If the worldwide emission pattern of CFC-12B1 follows that of other anthropogenic substances, it is reasonable to assume that $>90\%$ of the emission occurs in the Northern Hemisphere. If no hemispheric mixing were to occur, the increase of the abundance of CFC-12B1 observed at northern midlatitudes would correspond to a global annual release rate of 2.6×10^3 tons per year

However, as interhemispheric mixing occurs with a time constant of ~ 2 yr, the global release rate is likely to amount to $\sim 3.6 \times 10^3$ tons per year. Considering the error of $\pm 4\%$ related to the sensitivity correction of our analytical system, the range of this value may be $\pm 8\%$.

No values for the global production and emission rates of CFC-12B1 are available. For model studies, we have adopted an (arbitrary) scenario of exponential growth with global emission rates doubling every 18 yr. Scaling of these emission rates was performed such that the modelled profile, computed with a time-dependent one-dimensional model for 20 September 1982, matched the lower part of the observed profile (Fig. 1). Annual emission rates obtained in this way increased from ~ 1 kton per year in 1955 to 3.07, 3.23 and 3.34 kton per year for 1980, 1981 and 1982, respectively. Although the average annual emission rate of 3.6 kton per year derived from our measurements, for the 1983–84 period, seems to be consistent with this adopted emission scenario, the large relative increase of 20% per year observed for CFC-12B1 indicates that the doubling time of the annual worldwide emission rates is likely to be much shorter than 18 yr. The rapid growth of the atmospheric abundance of CFC-12B1 is also confirmed by near-surface measurements. While Rasmussen *et al.*⁸ report a value of 1.1 ± 0.2 p.p.t.v. measured at Barrow (Alaska) during 1983, a sample of fresh polar air collected on 5 February 1985 close to the MPAE site, yielded 2 p.p.t.v. of CFC-12B1. The abundances of CFC-11, CFC-12, CH_2Cl_2 and CCl_4 , which were analysed together with CFC-12B1, gave no evidence of local or regional pollution of this sample.

In the stratosphere, CFC-12B1 mixing ratios decrease by two orders of magnitude, to $\sim 10^{-14}$ at 25 km. The profile computed with the time-dependent model shows a slower fall-off with height, which reflects a well-known modelling problem related to source gases¹². Note that the rapid annual increase of CFC-

12B1 abundance is confined to heights below 20 km. In all three cases, radiosonde measurements from nearby meteorological stations revealed temperatures beginning to rise with height above 20 km. As stratospheric measurements of CO_2 indicate, substances from anthropogenic sources found above 20 km, in the height region of positive temperature gradient, have mostly penetrated through tropical upwelling, while the height region below 20 km is mostly governed by diffusion¹⁶. The related time lag for the 20–30-km layer with respect to the troposphere was found to be ~ 5 yr (ref. 15). This would confirm that the worldwide emission of CFC-12B1 into the atmosphere has been high in recent years.

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Transient electro-optic Raman scattering in liquid crystals

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We report here the first realization of time-resolved electro-optic Raman scattering in a liquid medium. Using the characteristic signal from a Raman active band, we show that an applied pulsed electric field induces a macroscopic particle reorientation which may be followed by the transient changes induced in the Raman signal. We have chosen a liquid-crystal material as the medium because this allows us to compare the static and dynamic response with other conventional techniques. This comparison indicates the foundation of a new method for characterizing orientational processes, and the dynamics thereof, at the local molecular structure level. The method does not require the use of perturbing dye or spin probes.

Many molecules and particles have anisotropic electric, magnetic and optical properties. Particles in fluid suspension, for example, seem to be optically isotropic because of the randomness of their individual orientation until an external field is applied. Such a field (which may be electric, magnetic, acoustic or optical) induces a partial alignment of the particles and the bulk material becomes optically anisotropic. Thus, because of the field-induced orientation, the bulk properties reflect the anisotropic properties of the individual particles. For such suspensions, electric-field induced changes in the optical properties (that is, scattered intensity¹, birefringence², dichroism², optical activity³ and fluorescence⁴) have led to the establishment of a series of standard techniques of 'electro-optic' characterization.

The use of pulsed fields, particularly, has allowed bulk molecular reorientation to be studied in these systems.

Liquid-crystal systems are, however, more complicated⁵. Structurally they have anisotropic electric, magnetic and optical properties but, because they are highly condensed systems, particle interactions and associations cause local domain structure on the size scale of a few micrometres. Unless suitably aligned, these domains are randomly orientated in space and cause an intense scattering of light giving rise to a milky or turbid appearance. If, however, these materials are contained in a thin cell and surface alignment techniques⁶ are used, they become optically clear and electro-optic phenomena may be observed. Such cells used with low molecular weight organic compounds and suitable optical arrangements form the basis of the liquid-crystal display industry⁷. Because of the condensed nature of the materials, only bulk properties are normally able to be studied and experimental data are referred to a 'director' which is essentially the mean direction or orientation within a domain. An applied electric field will cause director reorientation which will relax back to its original state on removal of the field. Unlike the simple non-interacting particle case, the orientation and relaxation processes now become dependent on the applied voltage, the elastic forces in the medium, as well as the dielectric properties. The situation is complicated because the molecules may not be rigid and, thus, intra-molecular relaxation could occur. Hitherto, the optical dynamics have been limited to relating the director motion to bulk material properties. Using the inherent chemical specificity of the Raman effect which arises from the vibrational modes of the polarizability ellipsoid associated with particular bonds, we can show that transient electro-optic methods may be used to follow the reorientation of the Raman tensor associated with a particular bond in the system.

We have assembled a novel Raman microprobe apparatus (see Fig. 1), based on an argon-ion laser used in the continuous

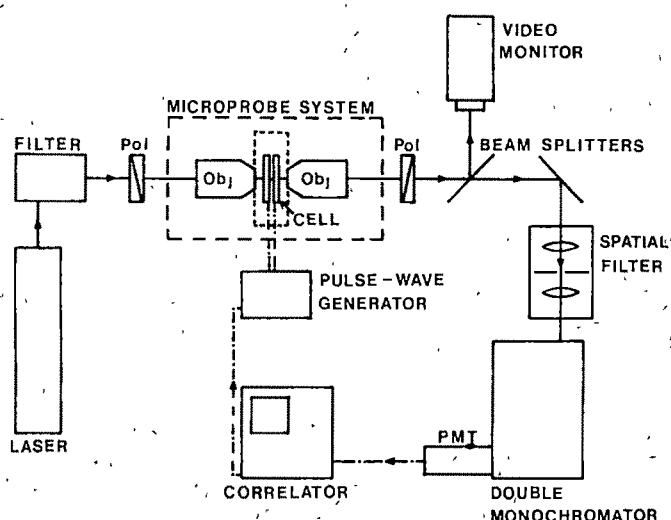


Fig. 1 A schematic diagram showing the Raman experimental arrangement.

wave mode at a wavelength, λ , 488 nm. To avoid spurious heating effects in the samples, the output power was kept below 20 mW. The output beam was then optically filtered to remove laser plasma lines and guided to the cell-microprobe assembly. This assembly used infinity-corrected microscope objectives ($\times 50$ magnification) and was custom built to include glan-laser polarizers (for controlling the polarization plane of the input and scattered light) and spatial filtering (to ensure that only the scattering from the sample volume was detected). The sample holder was an electrically heated constant-temperature oven stable to at least $\pm 0.1^\circ\text{C}$ and capable of holding microscope slides with X-Y translation. The scattered light was then collected in transmission (that is at zero scattering angle) using a further microscope objective ($\times 50$ again) and steered (using prisms and lenses to adjust the input light to the correct numerical aperture) to a Spex 1402 double-grating spectrometer ($f = 7.8$). Light from this spectrometer, which used high-resolution holographic gratings (1,200 grooves per mm), was then detected using a high-gain cooled photon-counting photomultiplier tube. The output signal from the photomultiplier was recorded using a 72-channel digital correlator operating in the signal-averaging mode.

The sample chosen for study was pentyl cyanobiphenyl (5CB), a highly stable liquid crystal exhibiting a nematic phase at room temperature, held in a cell constructed from two conducting glass slides. The cell surfaces were treated with a polyvinyl alcohol film which was then rubbed to give planar alignment⁷. In this way the director was aligned in a controlled manner in the cell and its direction, in the plane of the glass slides, aligned parallel to the input polarization of the argon-ion laser beam. The plane of the transparent glass electrodes was perpendicular to this beam. A voltage applied across the electrodes, therefore, produced a field in the direction of propagation of the beam. When the cell was inserted into the microprobe assembly, an area of $\sim 5 \mu\text{m}^2$ was illuminated. This was viewed using a video camera and monitor adapted to the collection optics. The video system allowed the condition of the sample to be examined at any time and thus allowed us to verify that any changes in the optical properties recorded were not due to heating or damage to the sample. The sample thickness (and optical path length) was $30 \mu\text{m}$ and a.c. voltages of between 2 and $6 V_{rms}$ were applied to the sample. For all the measurements reported here, the voltage was applied either continuously (for the steady-state response) or in 300–800 ms pulses (for the transient measurements).

With the director aligned in the polarization direction of the input light, the Raman active bands studied here gave a maximum response (see Fig. 2a). On application of an a.c.

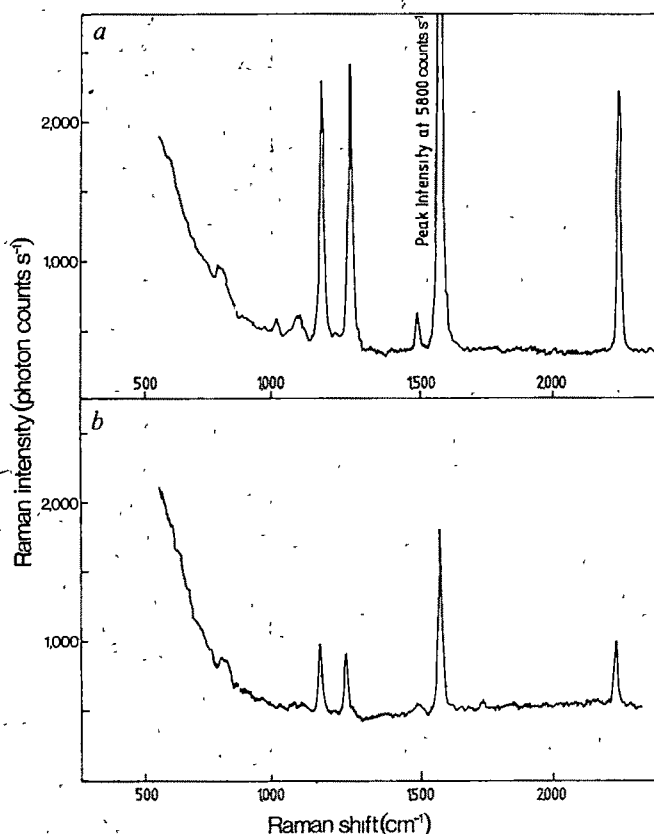


Fig. 2 Raman spectra for 5CB with: *a*, no applied field; *b*, an applied a.c. voltage of $2.2 V_{rms}$ at 500 Hz. Sample cell thickness was $30 \mu\text{m}$ and the polarization direction of both the input argon-ion laser beam and analyser for the scattered light was parallel to the director of the nematic phase. Temperature, 25.0°C . Numbers adjacent to the Raman lines correspond to the Raman shift from the exciting line at $\lambda = 488.0 \text{ nm}$. Assignments as follows: $1,179 \text{ cm}^{-1}$, C-H aromatic deformation; $1,285 \text{ cm}^{-1}$, C-C biphenyl link stretch mode; $1,607 \text{ cm}^{-1}$, C-C benzene ring stretch mode; $2,224 \text{ cm}^{-1}$, C $\equiv$ N cyano stretch mode. Increasing response at lower wavelengths marks the start of the wing of the Rayleigh scattering.

voltage across the cell, the liquid-crystal molecules orient according to their dipolar structure into the direction of propagation of the laser beam. The Raman tensors, therefore, rotate away from the polarization direction of the beam and the intensity of the Raman signal decreases (Fig. 2b). The strength of the Raman signals, with the steady-state field on, drop to about one-fifth of their field-off values. This reflects the high induced order in the system. On removal of the field, the spectrum relaxes back to its original field-off form. For both the field-on and field-off cases we have verified optically that the samples were not degraded.

Application of pulsed a.c. fields and the high sensitivity of the microprobe technique have allowed us to study time-dependent electro-optic Raman signals in these systems for the first time. A typical transient signal is shown in Fig. 3 for the $1,179 \text{ cm}^{-1}$ line. This response was recorded by monitoring the intensity (photon counts s^{-1}) at a fixed wavelength 517.8 nm (corresponding to this $1,179 \text{ cm}^{-1}$ line) as a function of time. From Fig. 3 it is clear that the field-on response time ($\sim 110 \text{ ms}$) is faster than the field-free decay ($\sim 400 \text{ ms}$). This is typical of such a liquid-crystal sample. In a normal liquid-crystal cell, the reorientational response time (τ_{on}) is a function of the applied voltage (V). For a planar-planar configuration, as used here, it would be expected, to a good approximation, that $\tau_{on} \propto V^{-2}$. We have measured τ_{on} as a function of V , Fig. 4, from which it can be seen that τ_{on} is indeed proportional to V^{-2} . Further, the field relaxation time (τ_{off}) should be independent of V . At

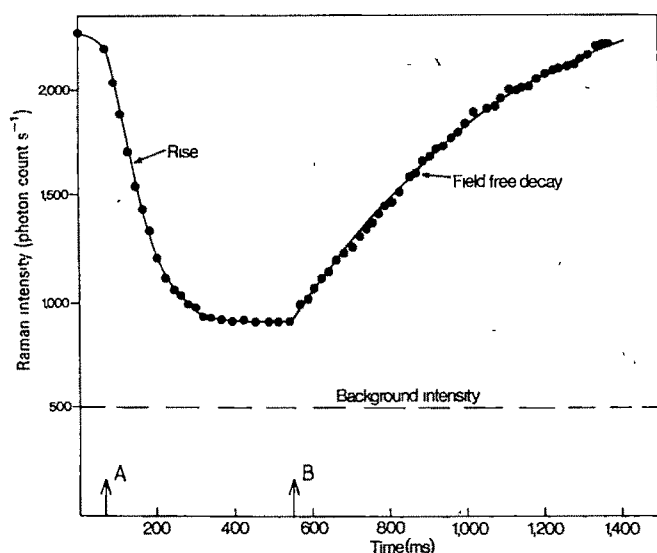


Fig. 3 Transient electro-optic Raman scattering in 5CB. Recorded signal corresponds to the $1,179\text{ cm}^{-1}$ Raman line. A and B denote the start and finish of the AC voltage pulse of amplitude $3.06 V_{rms}$ and frequency 500 Hz. Cell thickness was $30\text{ }\mu\text{m}$; temperature 25.0°C . The polarization conditions were as in Fig. 1.

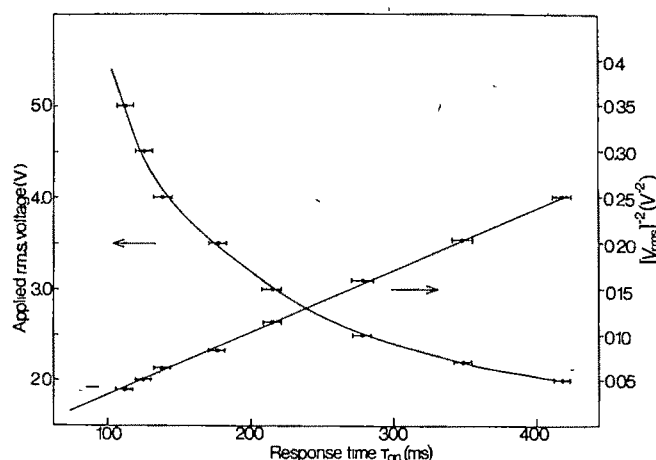


Fig. 4 Transient electro-optic response time (τ_{on}) as a function of the applied root mean square voltage (V_{rms}) and its inverse squared (V_{rms}^{-2}). Experimental conditions as in Figs 1 and 2 with the exception of the varied applied voltage. Transient response time is defined as the time for the Raman signal to fall to $1/e$ of the difference between the field-off and field-on saturation values.

constant temperature, τ_{off} was observed to be independent of V . Therefore, we concluded that the transient electro-optic Raman scattering (TEORS) is monitoring orientational motion and relaxation phenomena. The data presented refer to the weaker $1,179\text{ cm}^{-1}$ line which corresponds to a C-H 'deformation' mode in the aromatic ring structure of 5CB. Stronger signals were recorded for the other lines, particularly the $1,607\text{ cm}^{-1}$ C-C 'stretching' mode. However, we have chosen to use the weaker band to illustrate the potential of the TEORS method. All of the bands studied exhibited the same τ_{on} and τ_{off} characteristics. This is expected because these bands are all associated with chemical bonds located in the rigid core structure of the cyanobiphenyl molecule. Although we are now carrying out a detailed analysis of both the τ_{on} and τ_{off} data and applying the method to more flexible molecules, it is evident that the response times reported are typical for such liquid-crystal materials. We have verified this by studying the electro-optic response times

of the unshifted Rayleigh line using crossed polars in the same switching conditions.

We have established a new electro-optic method for characterizing orientational motion in fluid systems using the chemical selectivity of the Raman effect. Because of this selectivity, we do not need to use solvated dye or spin probes to study the orientational processes. If this field-driven technique is combined with the linewidth⁸ and order parameter⁹ methods already established for Raman scattering, then in one apparatus one has all of the experimental features normally only available to methods such as NMR. There are further advantages in that the experiment is much less expensive and allows rapid time dependent phenomena to be studied in real time. The sample volume is small ($\sim\mu\text{m}^3$) and, therefore, any feature that may be observed using a normal optical microscope may be studied in the Raman apparatus. Note that low concentrations of micrometre-sized pollutants have been studied by a time-averaged Raman microprobe system¹⁰. These experiments may now be carried out as a function of time.

We have recently applied our time dependent method to dilute solutions of polymers and polymeric liquid crystals and these results will be reported soon. We also envisage extending the studies to two areas of interest in the field of liquid crystals. The first is in the study of dye-liquid-crystal interactions in the so-called guest-host effect¹¹ where the dye molecules clearly perturb the local molecular dynamics, and the second is in the study of biological liquid crystals. Our original experiment was set up with the intention of studying the motion of proteins in lipid bilayers and cell walls so that the influence of drug additives on the protein dynamics might be monitored. Now that the technique has been established, both of these studies will be carried out and hopefully lead to a better understanding of the importance of local dynamics in such complex condensed systems.

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Dependence of the flexural rigidity of the continental lithosphere on rheology and temperature

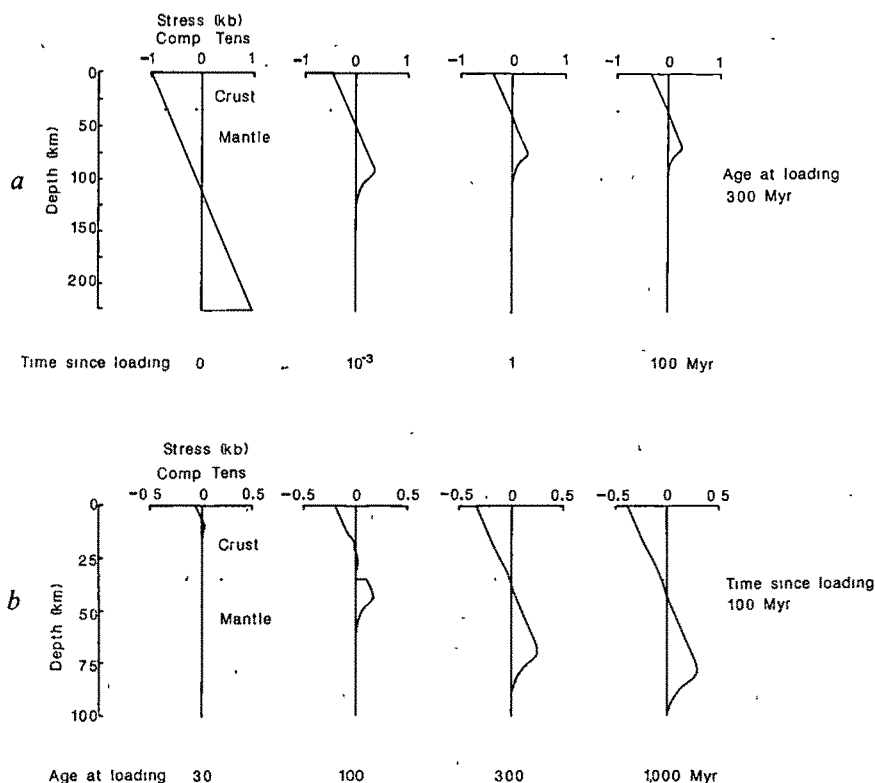
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While the first-order mechanical properties of oceanic and continental lithosphere are well defined and explain the observed increase in lithospheric flexural strength with thermal age, the temporal variation of continental flexural rigidity following loading, and its dependence on lithospheric composition and temperature structure, is only just beginning to be appreciated. We describe here the results of a quantitative rheological model in which laboratory-determined rock deformation data and transient

Fig. 1 Horizontal flexural stress plotted against depth: *a*, as a function of time elapsed since loading for lithosphere with a thermal age of 300 Myr; rapid stress relaxation is associated with a corresponding decrease in flexural rigidity; *b*, as a function of thermal age at time 100 Myr since loading. Increasing the thermal age allows the flexural stresses to be supported to a greater depth. In particular, Palaeozoic (300 Myr) and Proterozoic (1,000 Myr) lithospheres are associated with high flexural rigidities for Cenozoic (30 Myr) and Mesozoic (100 Myr) loading respectively.



temperature distributions, resulting from intra-plate basin formation within continental interiors, have been integrated. Continental flexural rigidity is shown to be controlled by crustal thickness, lithospheric thickness through the temperature structure, and the interaction of these factors during basin formation. For thermally young lithosphere, the flexural rigidity is dominated by the quartzofeldspathic rheology of the crust, while for the thermally older lithosphere, it is dominated by the olivine rheology of the mantle. Flexural data, through thermo-mechanical models of lithospheric deformation, provide a powerful constraint on both the transient and steady-state temperature structure of the lithosphere.

Studies of geological loading within the oceans and continents have established that lithospheric flexure is an important mechanism by which emplaced loads are supported¹⁻¹³. The flexural properties of the lithosphere can be parameterized by the flexural rigidity, D , which relates to the effective elastic thickness, T_e , by $D = ET_e^3/12(1-\nu^2)$, where E is Young's modulus and ν is Poisson's ratio. Geologically, T_e is the depth to the critical lithospheric isotherm which defines the elastic-plastic transition^{1,5}.

Two fundamentally different quantitative models have been developed to investigate the temporal mechanical properties of the continental lithosphere—the elastic and Maxwell visco-

elastic models—which predict fundamentally different time behaviours of the lithosphere following loading^{6,13}. The elastic model, as modified to reflect the thermal structure of the lithosphere at the time of loading (that is, the thermo-elastic model¹⁰⁻¹²), predicts deflections which are dependent only on the thermal age of lithosphere at the time of loading. In contrast, the Maxwell visco-elastic model is characterized by an initial high flexural rigidity which decreases with time following loading such that only the time since loading is important in defining the flexural response⁶. Flexural stresses created in a Maxwell visco-elastic lithosphere relax asymptotically to zero over geological time. As a consequence of their different time behaviour, the two models predict significantly different basin geometries and stratigraphies^{5,7,9,13}.

While the flexural deformation within the oceanic and continental lithospheres can be successfully analysed using the elastic model, unacceptably high elastic bending stresses² (10–50 kbar), rheological considerations of the way in which rock-forming minerals deform¹⁴, and the failure to predict the observed rapid relaxation from the seismic to an asymptotic elastic thickness¹, invalidates a purely elastic response of the lithosphere to loading. Alternatively, the Maxwell visco-elastic model in which low lithospheric rigidities are predicted 100–

Table 1 Age, load and rigidity parameters of various geological features

Geological feature	Age of load (Myr)	Thermal age of basement (Myr)	Thermal age of basement at time of loading (Myr)	Effective rigidity ($\nu = 0.25$, $E = 10^{12}$ dyn cm ⁻²)	Symbol in fig. 3
Lake Agassiz	0.007–0.014	1,600–1,800	1,600–1,800	5.9×10^{31} – 1.3×10^{32}	1 (ref. 6)
Lake Algonquin	0.00115–0.00125	1,200–1,500	1,200–1,500	2.9×10^{31} – 1.1×10^{32}	2 (ref. 6)
Ganges Basin	2–50	750–1,600	700–1,558	5×10^{31} – 10^{32}	3 (ref. 13)
Appalachian Basin	280–450	900–1,100	450–820	5×10^{31} – 2×10^{32}	4 (ref. 13)
Lake Bonneville	0.015–0.02	24–290	24–290	2.4×10^{29} – 2.9×10^{30}	5 (ref. 6)
Molasse Basin	2–40	270–365	230–363	10^{30} – 10^{31}	6 (ref. 13)
Anadarko Basin	230–330	510–530	180–300	4.1×10^{30} – 1.9×10^{31}	7 (refs 35, 36)
Michigan Basin	280–450	500–800	50–520	4.9×10^{30} – 1.2×10^{31}	8 (ref. 12)
Gulf Coast Basin	2–120	170–190	50–188	1.5×10^{30}	9 (refs 37, 38)
Williston Basin	113–351	500–600	149–487	7.2×10^{30} – 4.0×10^{31}	10 (ref. 31)

200 Myr after loading^{4,6} is equally inapplicable¹². A generalized model of continental lithospheric flexure is needed, therefore, which combines the long-term asymptotic finite rigidity of the thermo-elastic model with the short-term rapid relaxation of the Maxwell visco-elastic model. Such a model, using laboratory-derived creep deformation properties of quartz, feldspars and olivines, is termed a thermo-rheological model and incorporates the temperature and stress dependency of lithospheric material. This model has been used to examine quantitatively the relaxation of flexure-induced stresses with time as a function of depth, geothermal gradient (or lithospheric temperature structure) and crustal thickness within the continental lithosphere. The calculated stress-depth relationships are then used to determine the effective elastic thickness, T_e , and flexural rigidity of the lithosphere as a function of time, since loading.

Purely elastic initial horizontal flexural stress is assumed to take the form of $\sigma_x(t=0) = \sigma_0(1 - 2z/L)$ where z is depth, L the initial lithospheric thickness and σ_0 the maximum flexural stress before failure. The model assumes a visco-elastic Maxwell rheology for each infinitesimal component of the lithosphere, that is, for each component, $e_x = \sigma_x/E - v\sigma_y/E - v\sigma_z/E + e_x^v$ and similarly for e_y and e_z , where e_x is total strain, σ is stress and e_x^v is the viscous creep strain.

Conservation of horizontal force before and after flexure allows the additional constraint,

$$\int_0^L \sigma_x dz = 0$$

By assuming that the infinitesimal layers comprising the lithosphere are welded together during stress relaxation implies that

$$\frac{de_x}{dz} = 0$$

Manipulation of the above equation with the additional constraints of $\sigma_z = 0$ and $e_y = 0$ (plane strain) gives

$$\sigma_x = \int_0^t \left(\frac{1}{L} \int_0^L k \dot{e}_v dz - k \dot{e}_v \right) dt'$$

where $k = E/(1 - v^2)$ and

$$\dot{e}_v = (\sigma_x(2 - v) - \sigma_y(1 - 2v))/6\eta$$

where η is the apparent viscosity. The derivation is described in greater detail in ref. 15.

Temperature and power-law stress-dependent ductile deformation is assumed to be controlled by dislocation and Dorn law creep of olivine in the mantle¹⁶⁻¹⁸, while crustal creep is modelled as the dislocation creep of dry (lower crust) and wet (upper crust) quartz¹⁹. Dislocation creep takes the mathematical form $\dot{e}_v = A \exp(-E/RT) \tau^n$ where τ is shear stress, T is temperature (K) and A , E and n are material constants. The upper crust is assumed to contain 50% quartz and the lower crust only 10% quartz.

Stress modification by near-surface brittle failure of the crust has not been included in the model so that the primary thermo-rheological effects controlling flexure can be clearly demonstrated. Upper lithospheric brittle failure, when included in the model, results in only a minor reduction in flexural rigidity. Rigidity, therefore, as calculated here, represents an upper bound.

The lithospheric geothermal gradient has a critical role in controlling the strength of the lithosphere because of the temperature-dependent rheology of the crust and mantle. To span the range of acceptable geothermal models, geotherms have been calculated using cooling plate models²⁰⁻²⁵ with plate thicknesses of 125 and 225 km. Radiogenic heat productivity distributions given by Sclater *et al.*²³ and Nicolaysen *et al.*²⁴ have been included in the 125- and 225-km models respectively. An initial uniform plate temperature of 1,333 °C is assumed²⁵. With time, the lithosphere cools progressively by conduction so that the lithospheric temperature structure is directly related to its thermal age^{12,26}.

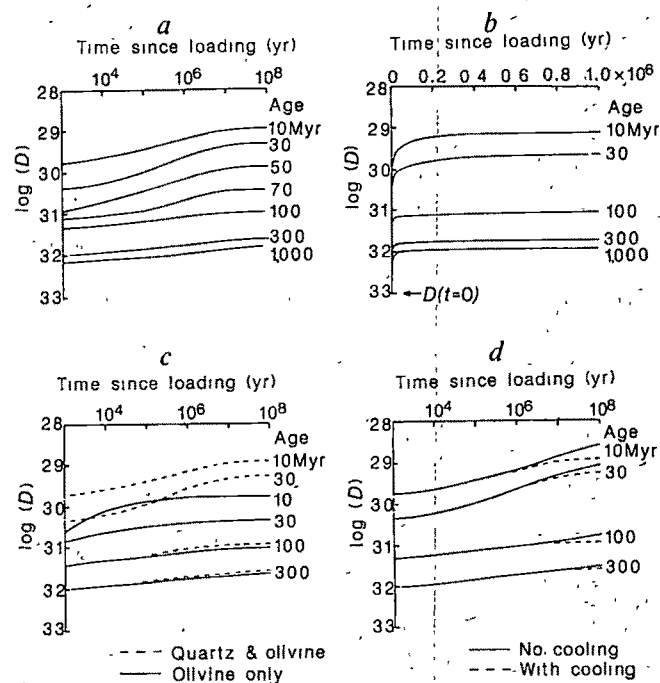
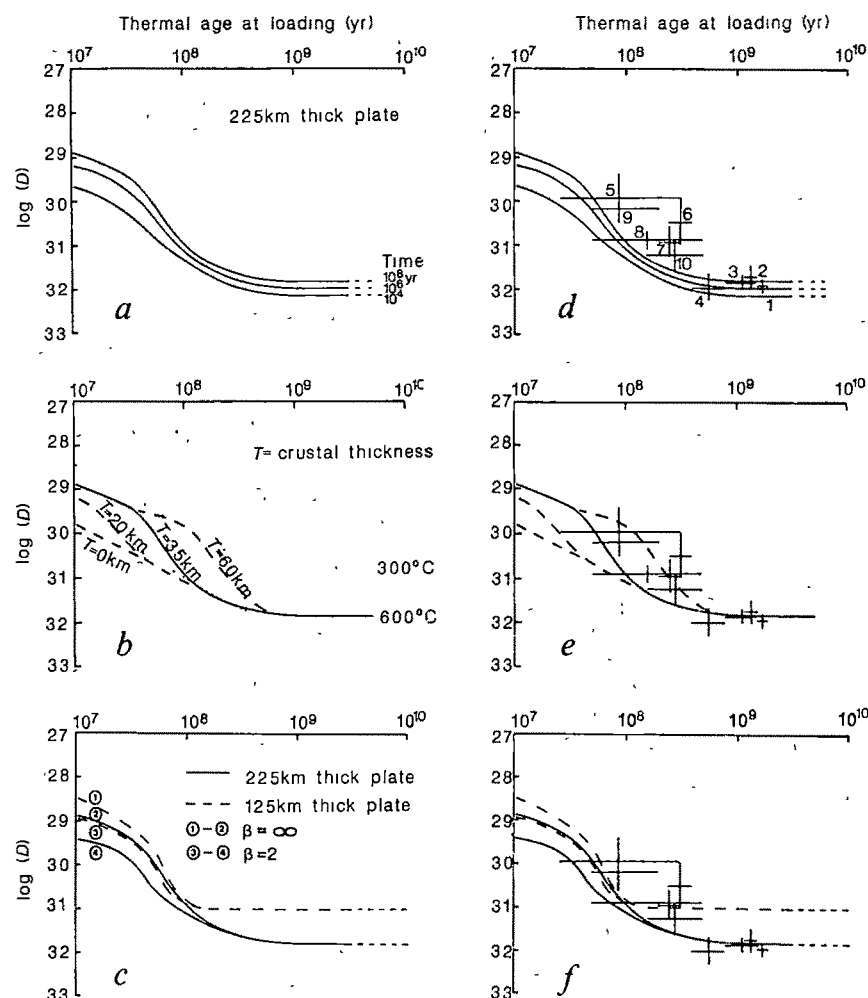


Fig. 2 The flexural rigidity, D : a, plotted against time since loading for lithosphere of various thermal ages with quartz rheology crust and olivine rheology mantle; the rate of relaxation towards the asymptotic rigidity increases with decreasing thermal age of the lithosphere; b, plotted against linear time; the rapid relaxation period, regardless of thermal age, is <0.2 Myr; c, compared with an all-olivine rheology lithosphere; the increase in the olivine component accelerates the flexural rigidity stabilization; d, compared with a model in which the geothermal gradient has remained constant since loading. This (secondary) effect is to reduce the value of the asymptotic rigidity. Lithospheric cooling therefore, leads to a more rapid stabilization of flexural rigidity by 'locking in' flexural stresses.

Figure 1a shows horizontal flexural stresses at various times after loading for lithosphere with a thermal age of 300 Myr (equivalent to Hercynian-aged lithosphere) and crustal thickness of 35 km. The initial flexural stress has a maximum compressive value of -1 kbar at the surface ($\sigma_0 = -1$ kbar). With time, ductile deformation results in stress relaxation within the lower lithosphere, reducing the depth extent over which the stresses are supported. Figure 1b shows the horizontal stress remaining after 100 Myr since loading for a range of lithospheric thermal ages. The older, hence colder, lithosphere shows stress supported to increasingly greater depths, corresponding to greater effective elastic thicknesses. Rheological variations associated with the Moho and at mid-crustal levels can be seen to generate discontinuities within the stress-depth relationships. The model described above conserves plate curvature during stress relaxation. An alternative condition in modelling flexure, more applicable to the lithospheric response to superimposed loads, would be the conservation of bending moment. For such a condition, stress relaxation in the lower lithosphere is accompanied by stress amplification in the upper lithosphere in order for the lithosphere to support the load. While the two models predict different stress levels, the resultant flexural rigidities are expected to be similar.

Rather than tracking lithospheric stress, it is advantageous to measure the variation in lithospheric flexural rigidity as it is this parameter which characterizes the surface deformation of the lithosphere. A simple estimate of flexural rigidity is obtained from the depth to the neutral fibre. In Fig. 2a, flexural rigidity is plotted against the logarithm of time since loading for lithospheric models with various thermal ages. An inverse relationship exists between flexural rigidity and the time since loading, whereas it increases with increasing lithospheric thermal age.

Fig. 3 Modelled flexural rigidity predicted by the 225-km plate thermo-rheological model as a function of the thermal age of the lithosphere at the time of loading: *a*, for elapsed times 10^4 , 10^6 and 10^8 yr after loading; *b*, showing the effects of crustal thickness variation; the predictions of the thermo-rheological model are compared with those of the thermo-elastic model for the 300 °C and 600 °C isotherms; *c*, compared with the predictions of the 125-km plate model; curves for stretching by $\beta = 2$ and $\beta = \infty$ (the oceanic plate cooling model) are shown, *d-f*, as for *a-c* but with superimposed observations of continental flexural rigidities as a function of the lithospheric thermal age at the time of loading.



For thermally young lithosphere, flexural rigidity rapidly stabilizes (within 10^6 yr) after loading, while thermally older and colder lithosphere takes longer. Lithosphere of 1,000 Myr thermal age exhibits a slow but continuous decrease in flexural rigidity with time. The plots of Fig. 2*a* using a logarithmic time axis are, however, misleading with respect to the rate of the initial relaxation phase. Figure 2*b* shows the time since loading plotted linearly against flexural rigidity and indicates that the rapid relaxation of flexural rigidity actually occurs within the first 0.2 Myr following loading, after which it slowly approaches its asymptotic value.

Comparison of the post-loading behaviour of the lithospheric rheological models with both composite quartz-olivine (crust-mantle) and all olivine compositions (Fig. 2*c*) indicates that an olivine lithosphere approaches its asymptotic flexural rigidity significantly more rapidly than a quartz-olivine lithosphere. This suggests that oceanic flexural rigidity should stabilize more rapidly than that of the continental lithosphere and, therefore, that it is flexurally stronger than continental lithosphere of the same thermal age.

A contribution to the stabilization of flexural rigidity arises from the continued cooling of the lithosphere following loading. Its significance is summarized in Fig. 2*d* which shows that the rate of rigidity stabilization is increased for oceanic lithosphere and thermally young continental lithosphere, both of which cool rapidly (it is this rapid cooling which essentially 'freezes in' the effective elastic thickness²⁷). However, this freezing is, in effect, of secondary importance relative to the main controlling factor on flexural rigidity stabilization, which is the stress decay within a material characterized by a temperature- and stress-dependent power-law rheology.

The flexural rigidity of the lithosphere tends to increase with its thermal age (Fig. 3*a*), a result which is consistent with flexural results from both the oceans and continents^{1,12}. Fig. 3*a* shows

separate curves for 10^4 , 10^6 and 10^8 yr after loading. The kink in the curves at 100 Myr results from the dual quartz-olivine rheology of the continental lithosphere. The contributions of the quartz and olivine rheologies are illustrated in Fig. 3*b*, where flexural rigidity is plotted against the thermal age of the lithosphere at the time of loading for models which consist of composite quartz-olivine (crust-mantle) and all olivine compositions. For thermally younger, hotter lithosphere, the flexural rigidity is controlled by the crustal quartz rheology while for older, cooler lithosphere (>200 Myr), the flexural rigidity is controlled by the olivine rheology. Flexural rigidity increases rapidly in the region of 100 Myr thermal age as the quartz rheological control gives way to that of olivine. As quartz has such an important role in lithospheric flexure^{12,28}, so must, therefore, the crustal thickness. Figure 3*b* shows the effect of changing crustal thickness ($T=0, 20, 35$ and 60 km) and, not surprisingly, increasing the crustal thickness tends to decrease the flexural rigidity of the continental lithosphere until control is again passed back to the mantle olivine component for large thermal ages. In Fig. 3*b*, the predictions of the rheological model are also compared with those of a simple thermo-elastic flexure model¹³ in which the effective elastic thickness is controlled by a critical isotherm defining the elastic-plastic transition. For thermally younger lithosphere, when quartz rheology controls lithospheric strength, the 350 °C isotherm is the critical isotherm while for thermally older lithosphere, when olivine rheology is more important, it is the 550 °C isotherm.

Many intracratonic basins of continental interiors are characterized by finite stretching of the lithosphere²⁹⁻³⁶ and, as such, will modify the temperature and hence mechanical structure of the lithosphere. Stretching in our context increases the surface heat flow by a factor β during the rifting process²⁹. In fig. 3*c*, the pre-rift lithosphere has a continental average heat flow²² of 60 mW m^{-2} . The resulting thermal perturbation is important in

defining the transient temperature structure of the continents. The variable spatial and temporal distribution of intra-continental rifting may, however, result in a complex temperature structure²², but with each rifting event, the thermal perturbation will decay by conductive cooling of the continental lithosphere regardless of previous history or spatial complexity. Figure 3c shows the relationship between the stretching factor responsible for resetting the continental lithospheric thermal structure and the corresponding (post-stretching/rifting) temporal variation in flexural rigidity for values appropriate to basin formation ($2 < \beta < \infty$). The effect of increasing β is to reduce the flexural rigidity early in the post-stretch period, the effect rapidly diminishing with time.

The temperature structure of the lithosphere is strongly dependent on its assumed thickness and radioactive distribution. To contrast the 225-km lithospheric thickness model (with the radioactive distribution of Nicolayson *et al.*²⁴—representing an upper bound) so far used, a 125-km lithospheric thickness model (with the radioactive distribution of Sclater *et al.*²³) has also been considered (Fig. 3c). The 125-km plate model predicts significantly lower flexural rigidities relative to the 225-km plate model for thermal ages $> 10^8$ yr.

The temporal variation in flexural rigidity of the continental lithosphere is strongly modified by crustal thickness (quartzic component), lithospheric thickness (olivine component and thermal time constant) and the interaction of these factors during basin formation (lithospheric stretching and transient temperature structure). Observations of continental flexural rigidity (for which both the age of the load and thermal age of the basement are known; Table 1), when plotted against the thermal age of their respective basements, are not compatible with any one thermal/lithospheric model (Fig. 3d–f). Realistic variations in crustal thickness and lithospheric stretching lead to modelled rigidities which at least help us to understand the scatter within the observations.

The loading of thermally old lithosphere ($> 10^8$ yr) is associated with essentially a steady-state lithospheric temperature structure. However, loading of thermally young lithosphere (the lithospheric temperature structure, therefore, being transient) results in considerable scatter in the observed rigidities which, in part, is related to either crustal thickness or lithospheric stretching variations (Fig. 3d, e). The remaining scatter is almost certainly a result of the inability to assess accurately the past or present thermal age of the continental lithosphere, either because of the failure to reset radiometric ages within the upper crust (or that the reset region has yet to be exposed by erosion) or possibly because radiometric age resetting lags significantly behind load reactivation (that is rigidity resetting) due to the thermal diffusivity of the lithosphere. For example, even though the Molasse Basin developed on Hercynian crust, as evidenced by recovered basement in Austrian and Swiss exploration wells, the apparently anomalous flexural rigidity for the basin (see Fig. 3a, Table 1) may reflect a bias introduced by thermal activity associated with either the formation of the Rhine Graben and/or major Tertiary–Quaternary volcanic activity within Europe (for example, Chaine des Puys, Kaiserstuhl, Vogelsberg and northern Bohemia).

If the temperature structure of the continental lithosphere is governed by a cooling plate model, a unique steady-state temperature structure for old lithosphere, and thus a unique asymptotic flexural rigidity, is a reasonable expectation. While variations in crustal radioactive heat production may produce significant heat flow anomalies, they have a negligible effect on sub-crustal lithospheric temperature and hence flexural rigidity. The grouping of reliable flexural rigidities for thermally old continental lithosphere is consistent with a plate model for the thermal evolution of the lithosphere and further, suggests a steady-state lithospheric thickness of 175–225 km.

Although our discussion began with flexural rigidity, it concludes with a statement on lithospheric temperature structure. Flexural data, therefore, through thermo-mechanical modelling of lithospheric deformation, provides a powerful constraint on

both the transient and steady-state temperature structure of the continental lithosphere. The thermo-elastic model^{1,2,12} satisfies the general thermal age dependency as indicated by flexural observations, but the detailed time evolution of flexural rigidity after loading, which controls the details of sedimentary basin geometry and stratigraphy, can only be addressed by a thermo-mechanical flexural model which integrates laboratory-determined rock deformation data and transient lithospheric temperature structures associated with either intracratonic sedimentary basin formation or continental collisions, such as presented here.

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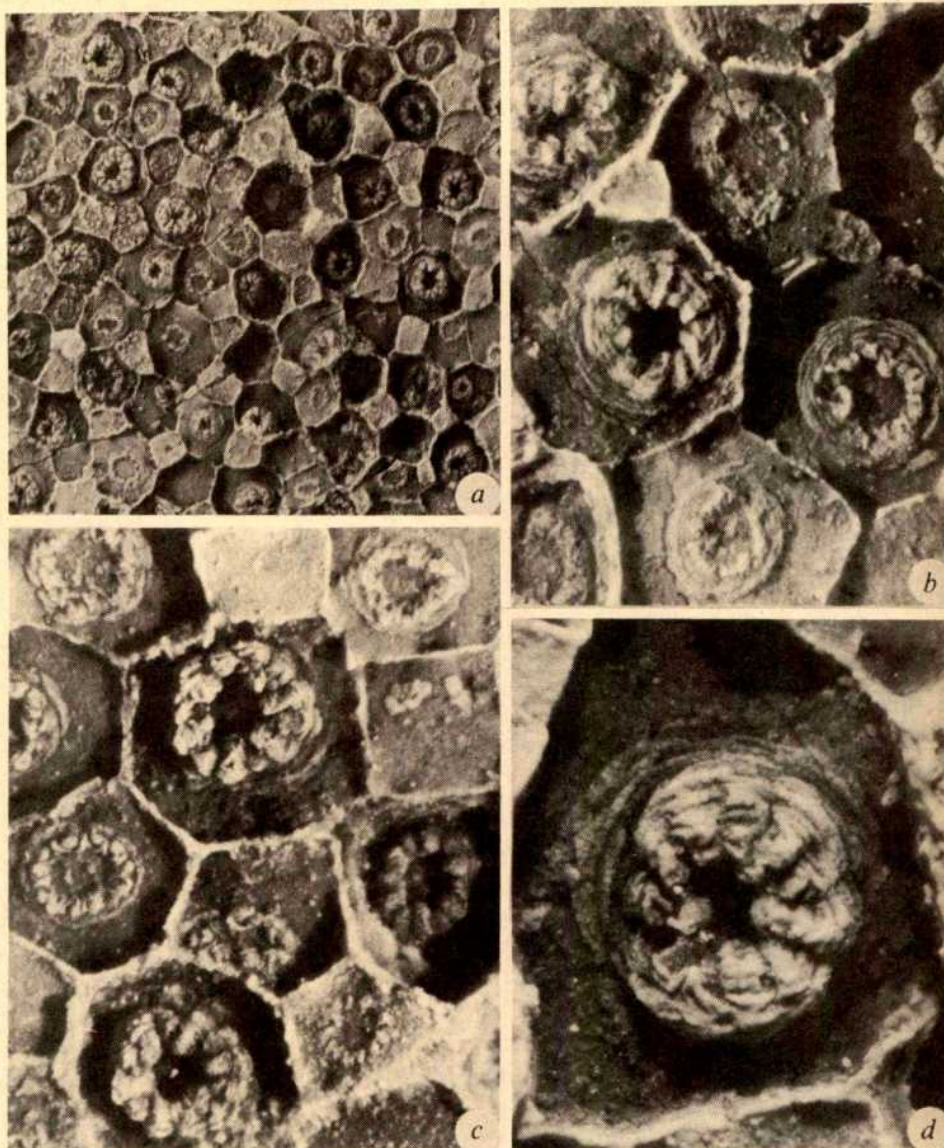
Fossilized polyps in 430-Myr-old *Favosites* corals

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Tabulate corals are among the more characteristic fossils of the Silurian period, but because only their skeletons have been preserved, their taxonomic position and mode of life have been uncertain. Although they are generally placed¹ in the phylum Coelenterata (Cnidaria), the recent discovery of living sclerospores that share architectural affinity with some tabulates, has renewed debate about the evolutionary relationships of the latter. In particular, the tabulates have been assigned^{2,3} to the sponges (phylum Porifera). I report here the discovery of fossilized polyps in a *Favosites* from the Silurian of Quebec. This incontrovertibly demonstrates that the tabulates belong to the phylum Cnidaria, and details of the soft-part morphology suggest that the Tabulata form a group distinct from other corals.

Fig. 1 Calcified polyp structures preserved in the calices of a *Favosites* corallum from the Jupiter Formation (Silurian, Llandoveryan) of Anticosti Island, eastern Canada (specimen GS79441, Geological Survey of Canada, Ottawa). *a*, Overview of the hemispherical colony showing 109 corallites, most with centred polyps ($\times 3.75$); *b*, *c*, detailed view showing varied stages of polyp degradation ($\times 12.75$); *d*, inwardly retracted tentacles and gut cavity and concentrically layered, radially striated view of the lower polyp stalk ($\times 33.75$).



Favosites, like most of the tabulate corals, secreted a skeleton of calcite, unlike the modern scleractinians which favour aragonite. Some modern anthozoans, such as sea anemones, produce sizable, 60-mm diameter, trochoid chitinous shells resembling gastropod conchs⁴. However, there seems to be no record of calcified, silicified or phosphatized polyps. Polyp tissues are soft and contain no apparent mechanism for *in situ* precipitation of calcium carbonate. This is produced only by the basal disk tissues that secreted the calicular wall, septa and tabulae. The discovery of unmistakable calcified polyps, therefore, is both surprising and exciting.

Six well-preserved coralla of previously undescribed species of *Favosites* were collected from the basal 50 m of the Jupiter Formation (early Silurian, Llandoveryan, C¹-C²) of Anticosti Island, Quebec, Canada. Materials came from two different localities: A55, NTS 12° E/11° W 74800:89170 and A98, 12° E/12° E 53760-820:34770-880 in the central and southern parts of Anticosti. The specimen illustrated in Fig. 1 comes from the second locality (A98), in the basal 10 m of the Jupiter, a coastal bluff section of richly fossiliferous calcareous shales with interbedded, thinly bedded, micritic limestones. The fauna includes the brachiopods *Eocoelia hemisphaerica sefinensis*, *Atrypa* (*Gotatrypa*), *Triplesia anticostiensis*, *Pentamerus oblongus*, *Leptaena*, and small matchstick bryozoans along with small, hemispherical *Favosites*. No other corals, stromatoporoids or calcareous algae were found. The fauna is consistent with a late Llandovery (C¹-C²) age. The graptolite *Monograptus sedgwicki*

has been found some 40 m higher in the section. The type locality for the boundary between the Jupiter Formation and the underlying Gun River Formation is adjacent to the discovery site.

The corallum showing the best polyp preservation is hemispherical and 6 cm in diameter. Corallite diameters vary from 1.5 to 2.5 mm, with mural pores in the centre of the walls, variably developed short septa and very thin corallite walls (as verified in thin sections). The calices were freed from enveloping sediment by immersing the corallum in Quaternary O for 1 h and cleaning in an ultrasonic bath for 80 min.

More than 200 of the corallites on the surface of the specimen in Fig. 1 showed the attachment of polyp structures preserved as calcite. Polyps were seen in a 'retracted' position, that is with the tentacular ring inwardly bent and tentacles inwardly directed. Tentacles tapered and were blunt-ended. Of more than 300 corallites measured in one corallum, ~70% had polyps preserved in varied stages of degradation from only the remnants of a basal disk, to those with shrunken tentacles, to fully displayed structures (Fig. 1). The fossilized polyps occupy about two-thirds of the calice: this is not significant because the fully extended polyps of living corals can be inflated to several times the corallite diameter. The concentric wrinkles at the base of the polyp stalk and fine radial elements similar to those seen in modern corals⁵ were also preserved (Fig. 1d).

The average number of individual tentacles measured for well-preserved polyps was 12; a few had 11 or 13 tentacles. No particular enlargement, division or symmetry of tentacles was

observed, suggesting that the tentacles were uniformly sized in *Favosites*, that is no 'sweeper' tentacles were indicated. The number of septal spines in the thin sections of *Favosites* examined was difficult to study as septal insertion was uneven or arrhythmic, and the septal spines were upwardly directed. However, *Astrocerium*, a close Silurian relative of *Favosites*, is characterized by 12 regular rows of septa. The Silurian heliolitid tabulates are also typified by 12 septa. The significance of the relationship between the discovery of 12-fold tentacles and the occurrence of 12 septa in several groups of tabulates is unclear. There may be no direct relationship.

The discovery of tentacular polyps in *Favosites* discards the possibility that the Favositidae, and probably other tabulates, are aberrant members of the Phylum Porifera, as has been suggested recently. Their taxonomic affinities seem to lie clearly with the Cnidaria.

The development of a common scheme of 12 tentacles for each polyp suggests the possibility of dodecal symmetry in the Favositidae. This, along with the known presence of 12 septa in other tabulates, seems to set the Tabulata away from the tetracorals (*Rugosa*), hexacorals (*Scleractinia*) and octocorals (*Octocorallia*).

The method of polyp calcification is as yet undetermined. In the absence of unusual environmental conditions on the stable marine carbonate platform of Anticost Island during the Silurian, it is tempting to suggest that algal symbionts were present in the tabulates and that these may have initiated precipitation of calcite nuclei or spicules inside the polyp. There is no evidence for this at present, as it is not clear whether calcification occurred while the polyp was alive or immediately after death. If algal symbionts were pervasive in the tabulates it is difficult to understand why polyp preservation is so rare.

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Paternal cyclophosphamide treatment of rats causes fetal loss and malformations without affecting male fertility

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The use of cytotoxic, mutagenic and carcinogenic agents as treatment for various types of cancer may be particularly hazardous in men of reproductive age as there exists the possibility that this may lead to congenital malformations in the progeny. Such agents can affect fertility and other aspects of male reproductive function, for example, treatment with anti-cancer drugs such as cyclophosphamide has been associated with oligozoospermia, azoospermia and increased levels of serum follicle-stimulating hormone (FSH)^{1,2}. Depending on the cumulative dose and the duration of treatment, spermatogenesis often returns but this may take years^{3,4}. The relevance of the effects of such chemicals on the male reproductive system to the offspring is poorly understood. We have set out to determine whether present tests of male reproductive function (that is, endocrine status, numbers of spermatozoa, fertility) can predict deleterious effects of a paternally administered agent on the offspring. Here, we report that chronic administration in rats

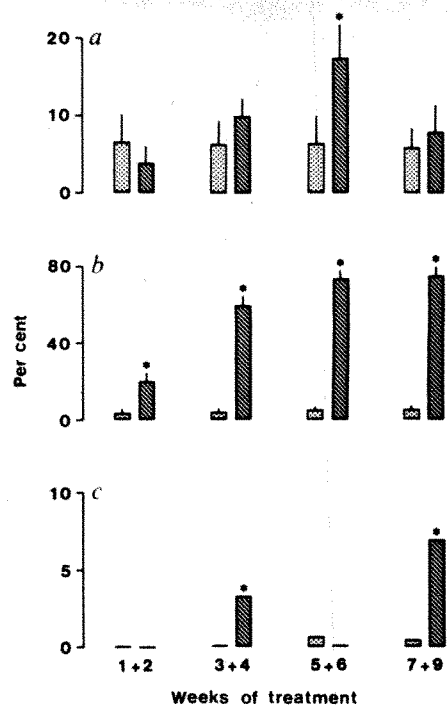


Fig. 1 Effects on the progeny from matings at specified times after initiation of paternal treatment with saline (□) or 5.1 mg per kg per day of cyclophosphamide (▨) by gavage. The mating schedule used allows an assessment of the stage of spermatogenesis affected by cyclophosphamide. Effects on the progeny after 1-2 weeks of treatment of the male indicate that epididymal spermatozoa were affected; similarly, after 3-4 weeks the germ cells affected were spermatids; after 5-6 weeks spermatocytes; and after 7-9 weeks spermatogonia⁷. Pre-implantation loss (a) is defined as the number of corpora lutea minus the number of implantations divided by the number of implantations. Post-implantation loss (b) is defined as the number of implantations minus the number of resorptions divided by the number of implantations. Both these values are expressed as per cent mean per litter. Fetuses were examined for gross malformations. External malformations (c) are expressed as the per cent of malformed fetuses divided by the total number of live fetuses for a given time period. Control and treatment groups were compared using a Fisher exact test¹⁰. The level of significance is taken as $P \leq 0.05$ throughout.

of low doses of the widely used drug cyclophosphamide had minimal effects on the male reproductive system and fertility, but resulted in malformations and retardation of growth in the surviving fetuses and a high frequency of fetal death. Thus, adverse effects on the fetus cannot be predicted from the effects of a drug on the male reproductive system.

Cyclophosphamide (CP) was chosen for this study as it is widely used clinically as an anti-cancer agent and has also been associated with variable periods of infertility in humans³. Adult male Sprague-Dawley rats were treated daily six times a week for 11 weeks by gavage with either saline solution (control) or one of three doses of CP (1.4, 3.4 and 5.1 mg per kg per day). These daily low-dose regimens simulated maintenance regimens used clinically for cancer treatment⁵. The 77-day treatment period covers one full cycle of the seminiferous epithelium (51.6 days) as well as the time needed for epididymal transit of the sperm (~10 days)^{6,7}. Thus, effects that are seen after the first 1-2 weeks of treatment reflect an action of the drug on spermatozoa undergoing maturation in the epididymis. Effects first seen 3-4 weeks after the initiation of treatment reflect action of the drug on spermiogenesis (spermatid development) or post-spermiogenesis changes, while effects first seen after 5-6 weeks of treatment indicate an effect on spermatogenesis or subsequent stages. An effect that selectively occurs 7-9 weeks after initiation of treatment would suggest strongly an effect on the proliferative spermatogonial phase of spermatogenesis.

The male rats were killed after 11 weeks and the effects of

Table 1 Effects of chronic treatment with cyclophosphamide for 11 weeks on the reproductive system of male rats

Treatment group	Body weight (g)		Tissue weight (g)		Sperm reserves ($\times 10^6$)*	Serum hormones (ng ml ⁻¹)		
	Initial	Final	Ventral prostate	Cauda epididymis		LH	FSH	Testosterone
Saline	317 $\pm$ 4	500 $\pm$ 14	0.57 $\pm$ 0.08	0.57 $\pm$ 0.02	152 $\pm$ 15	0.52 $\pm$ 0.06	193 $\pm$ 28	2.6 $\pm$ 0.6
Cyclophosphamide								
1.4 mg per kg per day	319 $\pm$ 5	534 $\pm$ 11	0.61 $\pm$ 0.06	0.52 $\pm$ 0.03	188 $\pm$ 17	0.43 $\pm$ 0.09	186 $\pm$ 28	2.2 $\pm$ 1.0
3.4 mg per kg per day	317 $\pm$ 3	481 $\pm$ 24	0.59 $\pm$ 0.05	0.59 $\pm$ 0.02	166 $\pm$ 15	0.36 $\pm$ 0.06	174 $\pm$ 21	1.8 $\pm$ 0.3
5.1 mg per kg per day	325 $\pm$ 4	484 $\pm$ 16	0.48 $\pm$ 0.04	0.59 $\pm$ 0.03	177 $\pm$ 17	0.38 $\pm$ 0.07	198 $\pm$ 13	2.4 $\pm$ 0.8

The serum concentrations of luteinizing hormone (LH), FSH¹⁸ and testosterone¹⁹ were measured by radioimmunoassay. For LH, NIH RP-2 preparations were used as standards. Numbers of spermatozoa in the cauda of the epididymis were determined by the method of Robb *et al.*²⁰ as modified by Scheer and Robaire¹⁹. Data were analysed by one-way analysis of variance with the new Duncan's multiple-range test²¹. Values are the mean $\pm$ s.e.m. The level of significance is taken as $P \leq 0.05$ throughout.

* Sperm reserves represent spermatozoal numbers in the cauda of the epididymis.

the drug treatment were assessed on the basis of body and reproductive organ weights, serum hormone levels and epididymal spermatozoal numbers. To assess the effects of the treatment on the progeny, each male ($n = 6$ per group) was mated overnight with two pro-oestrous females on day 7 of weeks 1 to 7 and 9 of treatment. Males were not treated with CP on the day of mating in order to preclude a direct effect of the drug on fertilization and post-fertilization events by transfer via the seminal fluid⁸. Females ($n = 384$) were killed 20 days after mating and caesarian sections were performed. The rate of embryo or fetal loss was determined by counting numbers of corpora lutea, implantations and resorptions, and the live fetuses were examined for external malformations.

The effects of the drug treatment on the male reproductive system of the rat are shown in Table 1. The similarities of initial and final body weights between the control and treated groups show that the treatment did not apparently affect the general health of the animals. There was no significant change in the weights of the male reproductive organs, in reserves of spermatozoa or in serum hormones reflecting male endocrine status in any of the CP-treated animals (Table 1). Ventral prostate and caudal epididymal weights were unchanged; testis, seminal vesicle and pituitary weights were also similar in the different groups (data not shown). As CP is an alkylating agent that causes most damage to rapidly dividing cells, the drug was expected to affect the seminiferous epithelium and reduce the number of spermatozoa produced. The number of spermatozoa in the cauda of the epididymis provides a good estimate of spermatozoal reserves⁹; clearly these reserves were not affected by the treatment regimen. These results suggest that the proliferative phase of spermatogenesis is unaffected by the cytotoxic effects of CP.

The mating ability and fertility of the males was similar in all groups, as assessed by counting seminal plugs on the morning after mating and the number of pregnant females per group. The number of plugs was similar for all treatment groups (in the range 3.6 ± 0.2 to 4.0 ± 0.2 ; mean $\pm$ s.e.m.). The number of pregnant females per sperm-positive females also did not differ between groups (91–97%). Clearly, the daily doses of CP used in these experiments did not affect the weight gain, endocrine status, spermatozoal reserves or the fertility of the treated males.

In view of the minimal effects of the CP treatment on the male reproductive system, the varied and complex effects on the progeny were quite unexpected. For clarity, only the results for the control and the group treated with 5.1 mg per kg CP are illustrated (Fig. 1). Results for the groups treated with 1.4 and 3.4 mg CP per kg fell between those for the control group and the groups treated with 5.1 mg CP per kg, in a dose-dependent fashion for all parameters. The control level of pre-implantation loss was relatively constant over the 9 weeks of mating (mean 7%, range 3–11%). Pre-implantation loss was low during the first 2 weeks of CP treatment, gradually increased between weeks 3 and 6, and was significantly ($P \leq 0.01$) greater than the control by weeks 5–6. However, this increase disappeared by weeks 7–9.

Thus pre-implantation loss occurred when germ cells were first exposed as spermatocytes or late spermatids.

There was a dramatic dose-dependent increase in post-implantation loss after week 2 ($P \leq 0.001$) for all animals receiving CP (Fig. 1); after week 4 a plateau of 80% loss was reached and maintained until weeks 7–9 ($P \leq 0.001$). The most striking increase in post-implantation loss occurred between weeks 2 and 4 when germ cells were first exposed as spermatids. To determine the timing of post-implantation loss, resorptions were weighed and defined as early (<100 mg) or late (>100 mg). Analysis of the weights of the resorptions revealed that this increase in post-implantation loss was due mainly to an increase in early resorptions; 87% of resorptions occurred early in gestation in the control group whereas 98% occurred early in the CP-treated group. Thus paternal treatment with CP resulted in germ-cell damage that was incompatible with development beyond organogenesis in a high percentage of embryos.

The CP-induced pre- and post-implantation losses resulted in a dose- and time-dependent decrease in the number of live fetuses per litter. While no increase of abnormalities was apparent in live offspring from matings at 1–2 weeks (control = 0/196, CP-treated = 0/184) and 5–6 weeks (control = 1/151, CP-treated = 0/55), the frequency of abnormalities was increased in offspring from matings at 3–4 weeks (control = 0/231, CP-treated = 3/93; $P < 0.02$) and at 7–9 weeks (control = 1/254, CP-treated = 4/57; $P \leq 0.005$). The most common abnormalities seen were hydrocephalus, micrognathia and oedema. There was also an increase in the percentage of growth-retarded or low-weight fetuses (fetuses weighing $<75\%$ of the mean weight for their group) in the offspring from matings at 3–4 weeks (control = 1.3%, CP-treated = 4.3%, $P \leq 0.10$) and at 7–9 weeks (control = 1.2%, CP-treated = 7.0%; $P \leq 0.02$, Fisher exact test¹⁰). (The dose of CP in all the above treatments was 5.1 mg per kg.)

Thus, paternal treatment with CP causes pre-implantation loss when germ cells were first exposed as spermatocytes or late spermatids. Post-implantation loss increased at the greatest rate when germ cells were first exposed as spermatids. There was a significant increase in external malformations and growth-retarded fetuses among the offspring when germ cells were first exposed as either spermatids or spermatogonia. Clearly, the low numbers of malformed fetuses involved make it difficult to elucidate the stage(s) of spermatogenesis affected. Different underlying mechanisms, both genetic and non-genetic, could be postulated to explain the effects, based on the fact that germ cells are first exposed to CP when they are undergoing different processes: spermatozoal maturation (1–2 weeks), cytoplasmic reorganization and chromatin condensation of spermiogenesis (3–4 weeks), meiosis during spermatocytogenesis (5–6 weeks) and active mitotic division (7–9 weeks).

Obviously, there are some severe qualitative changes in the spermatozoa of the treated males that are not manifested by concomitant quantitative changes. The effects seen are probably not due to a cumulative effect of CP in seminal fluid as: (1) the

drug has a short half-life in rodents and man and is eliminated within hours of ending chronic treatment^{11,12} (our protocol allowed 36 h between drug administration and mating); and (2) in the present study, there was no cumulative increase in pre-implantation loss and external malformations with increasing time of paternal drug exposure.

Previous studies in rats indicate that, after paternal treatment, CP can cause a decrease in litter size^{13,14} and lead to dominant lethal mutations¹⁵ as well as behavioural abnormalities in the surviving offspring¹⁴. Although the mechanism(s) underlying paternally mediated effects of CP on the outcome of pregnancy remains unresolved, one possibility is that this drug affects the genetic composition of the spermatozoa—CP has been shown to induce sister chromatid exchange and chromosomal aberrations in male germ cells^{16,17}. In addition, clinical studies have indicated that CP can affect the human male reproductive system¹. As far as we are aware, no study to date has attempted to relate effects on the male reproductive system to the outcome in the progeny. Thus, we have demonstrated, for the first time, that paternal treatment of rats with a commonly used antitumour drug can cause malformations and growth retardation of the surviving offspring and lead to dramatic embryo loss, while at the same time having minimal effects on the male's endocrine status or spermatozoal reserves.

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The J1 glycoprotein—a novel nervous system cell adhesion molecule of the L2/HNK-1 family

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The neural cell adhesion molecules L1 (refs 1, 2) and N-CAM (refs 3-5) share a common carbohydrate epitope that is recognized by the monoclonal antibodies L2 and HNK-1 (ref. 6). The L2/HNK-1 epitope is also present on the myelin-associated glycoprotein (MAG) which is thought to mediate surface interactions between the axon and myelinating cell⁷. Other, as yet unidentified,

cell-surface glycoproteins are recognized by the two antibodies and are believed to belong to a family of neural cell adhesion molecules^{1,2,6}. To test this hypothesis, we have prepared polyclonal antibodies to a prominent member of the L2/HNK-1 family, the 160K (relative molecular mass (M_r) 160,000) glycoprotein. Here we report that these antibodies, designated J1 antibodies, react with astrocytes and oligodendrocytes and interfere with neurone-astrocyte adhesion, but not with neurone-neurone or astrocyte-astrocyte adhesion. This result suggests the involvement of the J1 antigen in cell-cell interactions.

J1 antibodies do not react with L1, N-CAM or MAG (Fig. 1a-c), but react specifically with the 160K component of 'rest L2' (ref. 6; see Fig. 1 legend) from adult mouse brain (Fig. 1c). During cerebellar development the antigen is faintly detectable in Western blots of a crude membrane fraction at embryonic day 15, in the form of two faint bands with apparent M_r s of 220K and 200K (Fig. 1g). With increasing age, immunoreactivity shifts from the 220K and 200K bands towards the 160K band (Fig. 1h-k). In the adult form the 220K component is no longer present (Fig. 1c, l). When J1 antigen is isolated preparatively from 'rest L2' of adult brain, the 200K component is not detectable, possibly because of degradation during purification procedures. The J1 antigen is a glycoprotein, as it can be biosynthetically labelled with fucose (Fig. 1m) and is recognized by monoclonal antibody L2 (result not shown). Cultured astrocytes (Fig. 1n), but not neurones (Fig. 1o) purified from early postnatal cerebellum, synthesize the 220K form; this component is also secreted into the culture supernatant. In mixed cerebellar cultures the antibody reacts strongly on immunofluorescence with glial fibrillary acidic protein (GFAP)-positive astrocytes, with O4 and O1 antigen-positive oligodendrocytes (Fig. 2) and with some fibronectin-positive fibroblast-like cells (not shown). Neurones acquire faint immunoreactivity with time in culture, possibly resulting from adsorption of the secreted 220K form of the antigen. As the J1 antigen is secreted into the culture medium, we examined its possible relationship to characterized extracellular matrix proteins. Radioimmunoassays, however, fail to detect any significant reaction of J1 antibody with laminin⁸, collagen IV⁹, fibronectin, nidogen¹⁰ or heparan sulphate proteoglycan¹¹ (results not shown).

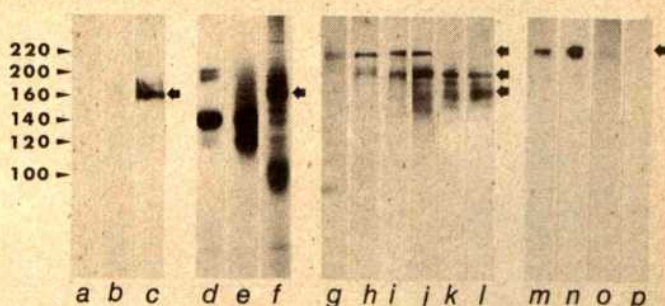
When adhesion of single-cell suspensions to monolayer cells is measured in the presence of Fab fragments of J1 antibody, neurone-astrocyte adhesion is inhibited, but not astrocyte-astrocyte or neurone-neurone adhesion (Table 1). The low level of adhesion between neurones and skin fibroblasts is also not disturbed to any measurable degree by J1 antibodies (not shown). That J1 antibodies do not interfere with adhesion of neurones to J1 antigen-positive skin fibroblasts could be the result of a molecular difference or a difference in cell-surface density of the J1 antigen between astrocytes and fibroblasts. Fab fragments of polyclonal antibodies to mouse liver membranes do not interfere with adhesion (Table 1), whereas polyclonal L1 antibodies prevent neurone-neurone adhesion, and polyclonal N-CAM antibodies prevent neurone-neurone, neurone-astrocyte and astrocyte-astrocyte adhesion (results not shown; see ref. 12).

The present experiments have revealed a novel neural cell adhesion molecule, the J1 antigen, which mediates neurone-astrocyte adhesion and may provide the molecular basis for the repeatedly observed preferential attachment of neuronal cell bodies to astrocytes, and the accelerated neurite extension on astrocyte membranes. As a secreted glycoprotein, J1 antigen may represent part of the extracellular matrix in the nervous system. As J1 antigen is synthesized by astrocytes and not by neurones, an adhesion mechanism between J1 glycoprotein(s) and a different receptor molecule(s) on neurones must be postulated. Whether L1 or N-CAM is involved in such ligand-receptor relationships, and whether J1 antigen contributes to the migration of granule cell neurones along the surface of Bergmann glial processes, remains to be seen.

J1 antigen is the third member of the L2/HNK-1 family of cell-surface glycoproteins to be examined for its involvement in

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Fig. 1 Immunochemical characterization of the J1 antigen. In Western blots, the J1 antibody did not react with immunopurified L1 (a) or N-CAM (b), and recognized the 160K component, but not the myelin-associated glycoprotein (MAG), the 100K component of 'rest L2' (c). Compare the Western blots (a-c) with the immunopurified L1 (d), N-CAM (e) and 'rest L2' (f) from adult mouse brain in reduced silver gels according to Merrill¹⁹. During cerebellar development, J1-immunoreactive material was examined by Western blot analysis of crude membrane fractions¹⁵ of C57BL/6J mice, after SDS-polyacrylamide gel electrophoretic separation on 6-12% gels with 200 µg protein per well, at embryonic day 15 (g), at postnatal days 0-2 (h), 4-6 (i), 8-12 (j) and 20-25 (k) and in the adult (>2 months old; l). m, J1 antigen is a glycoprotein, as it incorporates ³H-fucose into the 220K band; the lane shows an autoradiograph of an immunoprecipitate with J1 antibody. J1 antigen can be immunoprecipitated from cultures of pure astrocytes (>97% are positive for the astrocyte marker GFAP) as



as a strong 220K band (n) and as a very faint band from pure neurones (o) (>99% are positive for the neurone markers L1 antigen and tetanus toxin receptor) isolated from early postnatal mouse cerebellum as described elsewhere¹². The weak reactivity of the neuronal cultures is presumably caused by low contaminating levels of astrocytes and fibroblast-like cells. p, Autoradiograph of an immunoprecipitate from astrocytes reacted with preimmune rabbit antibodies. Arrows indicate the 160K, 200K and 220K components of J1 antigen.

Methods. Polyclonal antibodies were prepared in rabbits by injecting them with 160K glycoprotein isolated from adult mouse brain. The 160K band was purified from 'rest L2' (consisting of the L2 epitope-carrying molecules after removal of L1 antigen and N-CAM by immunoaffinity chromatography on monoclonal L1 and H28.123 (mouse N-CAM/BSP-2) columns⁶). 'Rest L2' was separated by chromatography on a Sephacryl S-300 column in Tris-buffered saline pH 8.2, containing 0.1% deoxycholate. The fractions containing the 160K glycoprotein were used for immunization. The pooled fractions were checked for purity in Western blots, which showed no reaction with polyclonal L1, N-CAM and MAG antibodies. On incubation with monoclonal L2 antibodies, the fractions showed mainly one broad band at 160K. This band was also the predominant one in SDS silver gels. Outbred rabbits were injected subcutaneously with 10 µg of purified 160K component in Tris-buffered saline, first in complete Freund's adjuvant and 2-3 weeks later in incomplete Freund's adjuvant. Seven days after the third intraperitoneal injection of 10 µg J1 antigen in Tris-buffered saline, rabbits were bled for serum. The purified IgG fraction of J1 antibodies was used in all experiments. ³H-fucose labelling was carried out in mixed cerebellar cultures¹⁶ from 6-day-old C57BL/6J mice (1×10^7 cells in a Petri dish of 6 cm diameter). After 3 days *in vitro*, cells were treated with ³H-fucose ($80 \mu\text{Ci ml}^{-1}$) in 1 ml of basal Eagle's medium containing 10% horse serum¹⁶ for 4 h in a CO₂ incubator, subsequently solubilized and used for immunoprecipitation as described previously¹². Immunoprecipitations of ³⁵S-methionine-labelled cells were carried out as described in detail elsewhere¹².

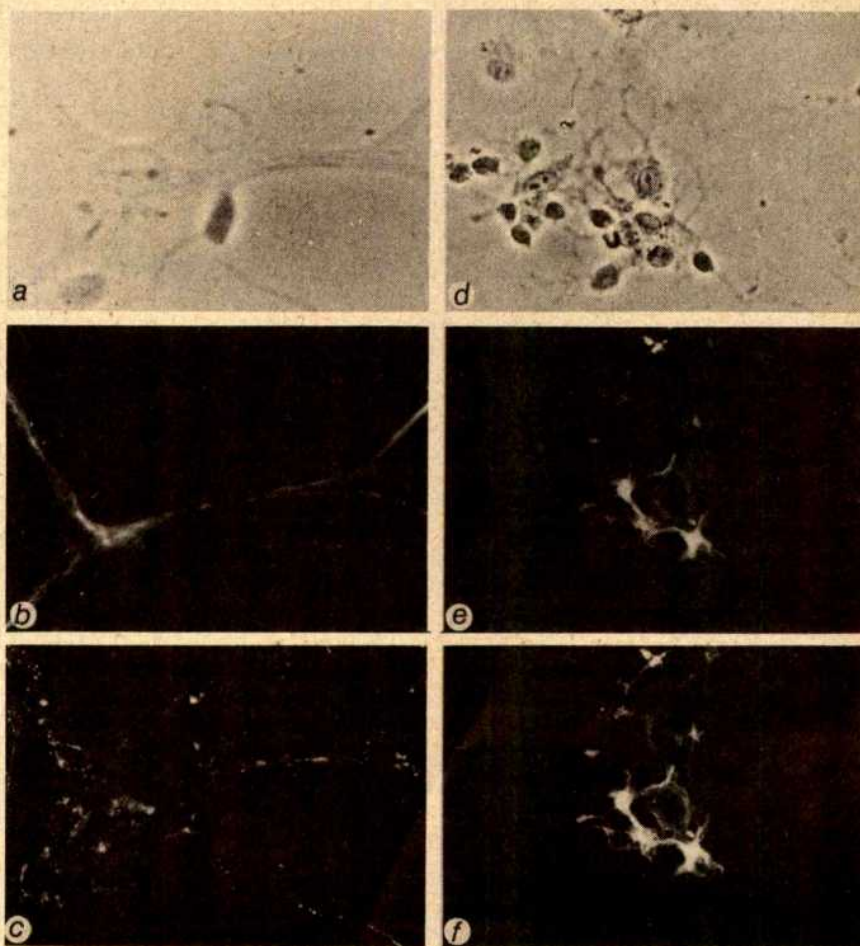


Fig. 2 Double immunofluorescence for J1 antigen (c, f) in monolayer cultures of 7-day-old C57BL/6J mouse cerebellum after 2 days *in vitro*; and for glial fibrillary acidic protein (b) and the oligodendrocyte marker O4 (e). a, d, Corresponding phase-contrast micrographs of the fluorescence images b, c and e, f, respectively. The procedures for cell culture and double immunofluorescence have been described elsewhere¹⁴⁻¹⁶. $\times 320$.

Table 1 Inhibition of adhesion between neurones and astrocytes from early postnatal mouse cerebellum in the presence of Fab fragments from J1 antibody

Antibody	Fab concentration (mg ml ⁻¹)	Neurone-neurone	Neurone-astrocyte	Astrocyte-neurone	Astrocyte-astrocyte
None	0	0 ± 3	0 ± 2	0 ± 4	0 ± 2
Polyclonal antibody to mouse liver	1	2 ± 4	-3 ± 2	-2 ± 3	1 ± 1
J1	1	-3 ± 8	47 ± 7	38 ± 15	-7 ± 12

The adhesion test was carried out as described elsewhere¹² using monolayer cultures as target cells and fluorescein diacetate-labelled single-cell suspensions as probe cells. Pure populations of astrocytes and neurones were obtained by buoyant density centrifugation of single-cell suspensions through Percoll, combined with immunocytolysis¹². Small cerebellar neurones were obtained from 6–8-day-old C57BL/6J mice and used as target cells after 3 days in culture, with over 99% of all cells expressing L1 antigen¹⁵ or tetanus toxin receptors¹⁶ and less than 1% expressing J1 antigen. Cerebellar astrocytes were obtained as described elsewhere¹² from 3–5-day-old mice by immunocytolysis with O4, MESA-1 and M5 antibodies in the presence of guinea pig complement, after 3 days of *in vitro* culture and subsequent subculture for 1 day; over 97% of all the cells expressed GFAP and more than 95% expressed J1 antigen. Fibroblasts were obtained from the skin of 1-day-old C57BL/6J mice by treatment with collagenase IV and trypsin¹⁷ and used as target cells after 3 days of *in vitro* culture. Over 90% of these cells expressed the J1 antigen. Probe cells were obtained from monolayer cultures of enriched neurones (after 1 day in primary culture), astrocytes (1 day after immunocytolysis and subsequent subculture) and fibroblasts (after 3 days in primary culture) by treatment with 20 µg ml⁻¹ trypsin, 1 mM EDTA in Ca²⁺, Mg²⁺-free Hank's balanced salt solution for 15 min at room temperature¹². Probe cells were labelled with fluorescein diacetate (10⁻⁶ M) during the trypsinization step. The mild trypsin treatment did not reduce the immunofluorescence staining intensities for either the J1 antibody or the antibody to mouse liver membrane¹⁸, as judged by comparison with non-trypsinized cells. Probe and target cells were treated with Fab fragments of J1 antibody for 20 min on ice before the adhesion test. Probe and target cells were then incubated for 30 min at room temperature in a reciprocal shaker at 40 cycles per min. Aggregation among probe cells was negligible in the presence or absence of Fab fragments. In the absence of Fab fragments the percentage of binding of total input cells was ~80% for neurone-neurone and neurone-astrocyte adhesion, 50% for astrocyte-astrocyte and astrocyte-neurone adhesion, and 10% for neurone-fibroblast adhesion (see ref. 12 for details). Per cent inhibition of adhesion in the presence of Fab fragments was calculated from:

$$\% \text{inhibition} = \left(\frac{\text{adhesion (control)} - \text{adhesion (+Fab)}}{\text{adhesion (control)}} \right) 100$$

Values are the mean of several experiments (±s.d.). Three experiments were performed for neurone-neurone adhesion, three for neurone-astrocyte adhesion, two for astrocyte-astrocyte, two for astrocyte-neurone and two for neurone-fibroblast adhesion. The difference in inhibition of adhesion due to liver membrane and J1 antibodies is significant for neurone-astrocyte and astrocyte-neurone adhesion ($P < 0.001$, Student's *t*-test).

cell-cell interactions, and indeed it has been shown to mediate adhesion. This result is in accord with our hypothesis that all L2/HNK-1-carrying molecules are functionally important in cell-surface interactions^{2,6}. The observation that further glycoproteins belong to the L2/HNK-1 family may now encourage and guide further search for new adhesion molecules displaying different sets of functional properties not only in the nervous system, but also in the immune system, where HNK-1 has been shown to react with a subclass of lymphocytes including natural killer cells¹³. The fact that all neural adhesion molecules identified so far share a carbohydrate moiety which is involved in adhesion¹², which appears to be developmentally regulated independently from the expression of the protein backbone of the adhesion molecule¹⁴, and which is differentially expressed on some, but not all N-CAM molecules⁶, indicates an important functional role¹² that is highly conserved among vertebrate species and which has yet to be elucidated in molecular terms.

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Pertussis toxin reverses adenosine inhibition of neuronal glutamate release

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Adenosine and its analogues are potent inhibitors of synaptic activity in the central and peripheral nervous system^{1,2}. In the central nervous system (CNS), this appears to arise primarily by inhibition of presynaptic release of transmitters^{3,4}, including glutamate⁵, which is possibly the major excitatory transmitter in the brain. In addition, postsynaptic effects of adenosine have been reported which would also serve to reduce neurotransmission^{6,7}. The mechanism by which adenosine inhibits CNS neurotransmission is unknown, although it appears to exert its effect via an A₁ receptor^{2,8} which in some systems is negatively coupled to adenylate cyclase⁹. In an attempt to elucidate the mechanism of inhibition, we have examined the effect of pertussis toxin (PTX) on the ability of the stable adenosine analogue (–)-phenylisopropyladenosine (PIA) to inhibit glutamate release from cerebellar neurones maintained in primary culture. PTX, by ADP-ribosylating the nucleotide-binding protein N_i, prevents coupling of inhibitory receptors such as the A₁ receptor to adenylate cyclase¹⁰. As reported here, we found that PTX, as well as preventing inhibition of adenylate cyclase by PIA, also converts the PIA-induced inhibition of glutamate release to a stimulation. Our results suggest strongly that purinergic inhibitory modulation of transmitter release occurs by inhibition of adenylate cyclase.

Although previous experiments concerning the effects of adenosine on transmitter release from brain tissue have been performed on slices or synaptosomes, our preliminary results indicated that incubation of hippocampal slices (300 µm thick) for 1 h with PTX (0.1–1.0 µg ml⁻¹) had no effect on the ability of the adenosine analogue 2-chloroadenosine to inhibit trans-

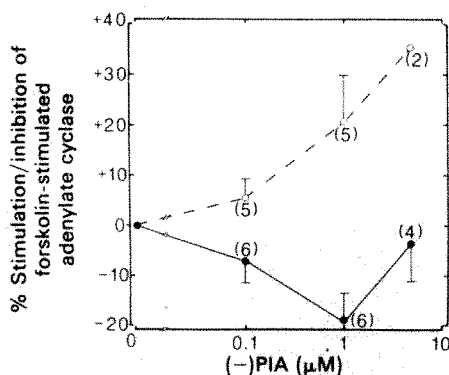


Fig. 1 Effect of incubation of cultured cerebellar neurones with PTX on (-)PIA-induced inhibition of adenylyl cyclase in membranes prepared from the cells. We investigated the effect of (-)PIA on the stimulation of adenylyl cyclase by a submaximal concentration of forskolin (2 μM). The basal adenylyl cyclase activity was 8.3 ± 3.5 and 8.9 ± 2.7 pmol per min per mg protein and the forskolin-stimulated activity was 14.7 ± 2.4 and 33.3 ± 8.8 pmol per min per mg protein in control (●) and PTX-treated (○) cells respectively (mean $\pm$ s.e.m.). Numbers in parentheses indicate number of experiments.

Methods. Rat cerebellar neurones were grown on poly-L-lysine in 35-mm Petri dishes for 13–21 days, having been treated with fluorodeoxyuridine (80 μM) after 2 days *in vitro* to reduce non-neuronal cell proliferation. They were incubated for 14–16 h at 37 °C with either PTX (0.5–1.0 μg ml⁻¹) or its vehicle (1 M NaCl, 10 mM Tris-HCl pH 8, diluted 100-fold) in 0.5 ml of growth medium (minimal essential medium (MEM), 10% fetal calf serum, 25 mM KCl). The cells were rinsed with buffer and collected in 10 mM Tris-HCl, 2 mM EGTA pH 7.4. After homogenization, the membranes were washed twice and adenylyl cyclase activity was assayed as described in ref. 13, except that EGTA (0.5 mM) was present in the final incubation medium and incubation was continued for 20 min.

mitter release (A.C.D. and E. R. Archer, unpublished data). As incubation of slices for longer periods markedly decreased the ability of the slices to synthesize and release glutamate, the effect of incubation with PTX for longer time periods was not examined. Instead, we examined the effects of PTX in a cell culture system. Cerebellar cells cultured from 3–6-day-old rats consist mainly of granule cells and have previously been found to release glutamate on depolarization, in a Ca²⁺-dependent manner¹¹. The neurones were grown, as described previously^{11,12}, on coverslips for the transmitter release studies (for method see Fig. 2 legend). For each experiment, we determined the amount of newly synthesized ³H-glutamate released in two periods of depolarizing stimulation (S₁ and S₂; see Fig. 2 legend). The S₂/S₁ ratio was then compared between experiments.

In adult animals, A₁ adenosine receptors have been shown to be associated with cerebellar granule cells¹³; in agreement with this observation, in our initial studies we found that inhibition of ³H-glutamate release by (-)PIA, which is a relatively selective A₁ agonist, developed at the same time as stimulus-dependent transmitter release became maximal, between 7 and 12 days *in vitro*, depending on the batch of neurones. For example, between days 7 and 9 *in vitro*, stimulation with 50 μM veratridine caused release of $1,553 \pm 332$ d.p.m. ($n=6$) over basal levels of ³H-glutamate and (-)PIA (2 μM) inhibited release by $12.1 \pm 1.2\%$ ($n=3$), whereas between days 14 and 21 *in vitro*, the same stimulus resulted in release of $2,860 \pm 713$ d.p.m. ($n=8$) of ³H-glutamate and inhibition by (-)PIA was $57.0 \pm 13.1\%$ ($n=4$). In subsequent experiments, neurones were used between days 13 and 21 *in vitro*.

When cultured neurones were incubated with PTX for 14–16 h, the inhibition of adenylyl cyclase by (-)PIA seen in washed membranes prepared from a cell-free preparation of control cells (preincubated with vehicle) was converted to a marked stimulation (Fig. 1). This result not only indicates that in these cells PTX is effective in preventing negative coupling to adenylyl cyclase, in parallel with its effect in other sys-

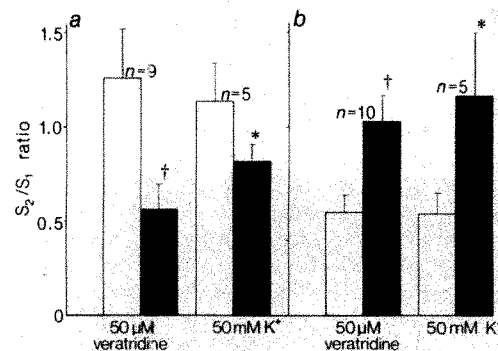


Fig. 2 Effect of PTX on the inhibition by (-)PIA of ³H-glutamate release from cultured cerebellar neurones. The amount of ³H-glutamate released after stimulation was calculated by subtracting the averaged basal release, and the ratio S₂/S₁ was determined for each experiment. A pair of control (open columns) and (-)PIA (solid columns) experiments were performed on the same day on the same batch of neurones, and the significances of the differences between their S₂/S₁ ratios (given as mean $\pm$ s.e.m.) were determined by paired t-tests. * $P < 0.05$; † $P < 0.01$. The outflow of ³H-glutamate (in d.p.m.) during S₁ for control (a) and PTX-treated (b) neurones was, respectively, $4,899 \pm 1,452$ and $5,423 \pm 1,410$ for K⁺ stimulation, and $1,979 \pm 264$ and $2,868 \pm 509$ for veratridine stimulation. The increases due to PTX treatment did not reach statistical significance.

Methods. Neurones (13–21 days *in vitro*), grown on 1-cm² coverslips, were preincubated for 14–16 h at 37 °C with PTX (0.5–1.0 μg ml⁻¹) or its vehicle in 100 μl growth medium. They were then rinsed and incubated with ³H-glutamine (5 μCi in 100 μl of Krebs-Ringer buffer (KRB) pH 7.4; 320 mM) for 45 min. The coverslips were rinsed and transferred to a perfusion chamber¹² and perfused with KRB at 35 °C at a rate of 1 ml min⁻¹. Two periods of stimulation were applied 18 min (S₁) and 36 min (S₂) after the start of perfusion, with either 50 mM K⁺-KRB for 4 min or 50 μM veratridine-KRB for 3 min. To examine the effect of (-)PIA (2 μM), it was included in the KRB from 27 min after the start of perfusion. Fractions collected at 1-min intervals were placed in tubes containing 10 mM each of carrier glutamine, glutamate and γ-aminobutyric acid (GABA) and 1.5 nCi of ¹⁴C-glutamate to correct for recovery. The ³H-glutamate in the fractions was separated from labelled glutamine and GABA by column chromatography as described previously⁵.

tems^{14,15}, but also that PTX treatment allows the expression of an underlying stimulation of adenylyl cyclase, possibly via A₂ receptor activation.

We then examined the effect of PTX on the ability of (-)PIA to inhibit depolarization-induced release of newly synthesized glutamate. In control experiments in the absence of (-)PIA, PTX altered the pattern of release of ³H-glutamate, in that the S₂/S₁ ratio was reduced in PTX-treated compared with control cells (Fig. 2). It is possible that the enhanced release of ³H-glutamate in S₁ relative to S₂ in PTX-treated cells results from either an increased concentration of endogenous cyclic AMP or the inability of endogenously released adenosine to inhibit glutamate release in PTX-treated cells. Because of the finite amount of ³H-glutamate available for release, there would be a corresponding reduction in S₂ and thus a reduction in the S₂/S₁ ratio.

We observed a more marked effect of PTX treatment on the response to PIA. Whereas in control cells (-)PIA (2 μM) inhibited both veratridine- and K⁺-stimulated release of ³H-glutamate by 55 and 28%, respectively (Fig. 2a), in PTX-treated cells there was a marked enhancement of ³H-glutamate release in the presence of (-)PIA (Fig. 2b), by 87 and 117% for veratridine and K⁺ stimulation, respectively.

The conversion of (-)PIA-induced inhibition of ³H-glutamate release to a stimulation by pretreatment with PTX mirrors the effect of PTX on adenylyl cyclase in these cells. In addition, in control intact cerebellar neurones, synthesis of cyclic AMP is enhanced by the depolarizing stimuli used, and PIA inhibits both control and K⁺-stimulated cyclic AMP production (A.C.D. and S.A.P., preliminary results). We suggest, therefore, that the

mechanism by which adenosine analogues inhibit presynaptic neuronal activity involves inhibition of adenylate cyclase. Furthermore, stimulation of adenylate cyclase by (-)PIA in PTX-treated cells may be responsible for the observed enhancement of transmitter release. Previous studies have suggested that selective A₂ receptor activation and augmented cyclic AMP levels enhance transmitter release^{5,15}.

The ionic mechanism associated with inhibition of synaptic activity by adenosine analogues acting at A₁ receptors remains a matter for speculation. Adenosine analogues have been found to inhibit synaptosomal ⁴⁵Ca fluxes¹⁶, whereas in an electrophysiological study they had no apparent effect on voltage-sensitive Ca²⁺ currents in hippocampal CA1 cells¹⁷. However, in neuroblastoma¹⁸ and rat dorsal root ganglion cells¹⁹, Ca²⁺ currents were potentially inhibited by adenosine analogues. It is possible that different mechanisms exist for pre- and postsynaptic effects of A₁ receptor stimulation.

In several systems PTX has been used to distinguish between receptors which are negatively coupled to adenylate cyclase, and those which are termed calcium-mobilizing, on which PTX had no effect^{20,21}. However, in mast cells PTX has been reported to block the secretory process by preventing activation of calcium-mobilizing receptors²². As neither A₁ nor A₂ adenosine receptors have been classified in this way²³, and as in the present study PTX enhanced rather than inhibited transmitter release, it is unlikely that it acts on calcium-mobilizing receptors. Thus, the results presented here provide clear evidence for the involvement of the N_i protein in the inhibitory effect of A₁ receptor stimulation on transmitter release; it can thus be classed in the same functional group of presynaptic neuronal receptors as enkephalin and α₂ receptors²⁴.

This work was supported by the MRC. We thank Bob Russell and Eric Archer for technical assistance, Drs L. Irons and A. Robinson (PHLS-CAMR, Porton Down, UK) for providing the pertussis toxin, and Dr B. Pearce for the perfusion chamber. **Note added in proof:** In recent experiments we have found (-)PIA (0.5–5 μM) to have no effect on either basal or K⁺-stimulated accumulation of inositol mono-, bis- or trisphosphates, in cerebellar neurones in culture, supporting our contention that (-)PIA acts by inhibition of adenylate cyclase.

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Activated human eosinophils generate SRS-A leukotrienes following IgG-dependent stimulation

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Eosinophils, a class of granular leukocytes, are prominent in many inflammatory processes, particularly in asthma, certain allergic diseases and during infections with helminthic parasites. Following incubation with the Ca ionophore A23187 (refs 1–4) (a non-physiological agent which circumvents membrane calcium-gating mechanisms⁵), eosinophils generate large amounts of sulphidopeptide leukotrienes, potent inducers of smooth muscle constriction^{6–9} and mucus production¹⁰. These are now known to represent the activity previously termed 'slow-reacting substance of anaphylaxis' (SRS-A) but attempts to identify a physiological stimulus for SRS-A production by eosinophils have so far been unsuccessful. The cells contain recognized receptors for IgG (Fc)^{11–14} and it is known that they adhere to, and can be activated by, contact with the surface of large organisms such as helminthic larvae¹⁵. We show here that eosinophils, particularly when activated, produce sulphidopeptide leukotrienes after contact with large particles coated with IgG.

We found that normal eosinophils generated appreciable amounts of SRS-A leukotrienes when incubated with IgG-coated particles, and that this was enhanced twofold when cells were stimulated *in vitro* (by the bacterial analogue f-Met-Leu-Phe (fMLP)) and up to 10-fold when activated low-density cells (from patients with hypereosinophilia) were studied.

Evidence for a harmful role for eosinophils in asthma is

provided by the observation that one of the granular constituents, major basic protein, is released onto and damages bronchial epithelium^{16–19}. On the other hand, this mechanism might give the eosinophil a protective function in adaptive immunity to certain parasitic diseases^{20,21}.

A23187-dependent eosinophil generation of sulphidopeptide leukotrienes (LTC₄, LTD₄, LTE₄)^{1–4} provides a theoretical mechanism by which eosinophils could contribute to the immediate asthmatic reaction. However, the physiological relevance of this observation has remained uncertain because of the artificial nature of the stimulus. Despite evidence of generation of leukotrienes from other circulating cell types using more pathophysiological agents (that is, LTB₄ from neutrophils by opsonized zymosan²², LTB₄ and LTC₄ from mononuclear cells by unopsonized zymosan²³ and LTC₄ from alveolar macrophages by IgE complexes²⁴), no comparable trigger for eosinophil sulphidopeptide leukotriene generation has been identified.

Eosinophils from patients with hypereosinophilia have an increased capacity to kill helminths²⁵. Many of these cells are hypogranular and have reduced density^{26–28}. These low-density cells have altered oxidative metabolism^{29,30}, an enhanced capacity to kill schistosomula of *Schistosoma mansoni* *in vitro*³¹ and increased expression of IgE (Fc) receptors^{32,33}. In addition, low-density eosinophils have an altered expression of cell-surface antigenic determinants³⁴ and are thought to be a sub-population of 'activated' cells.

In the present study IgG-coated Sepharose beads, which are 5–10 times larger than eosinophils (50–100 μm in diameter compared with 14 μm), were used as a stimulant, and the capacity of low-density and normal-density eosinophils to generate sulphidopeptide leukotrienes was examined. Incubation of normal-density eosinophils with IgG-coated beads resulted in a time-dependent release of these leukotrienes (Fig. 1b) related to the amount of adherent IgG (Fig. 1a) and not found with beads coated with human albumin. Furthermore, leukotriene generation by eosinophils in the presence of IgG-coated beads was significantly enhanced by prior incubation with fMLP (10⁻⁸ M) for 30 min (*P* < 0.02) (Fig. 1a, b). Incubation with fMLP in the

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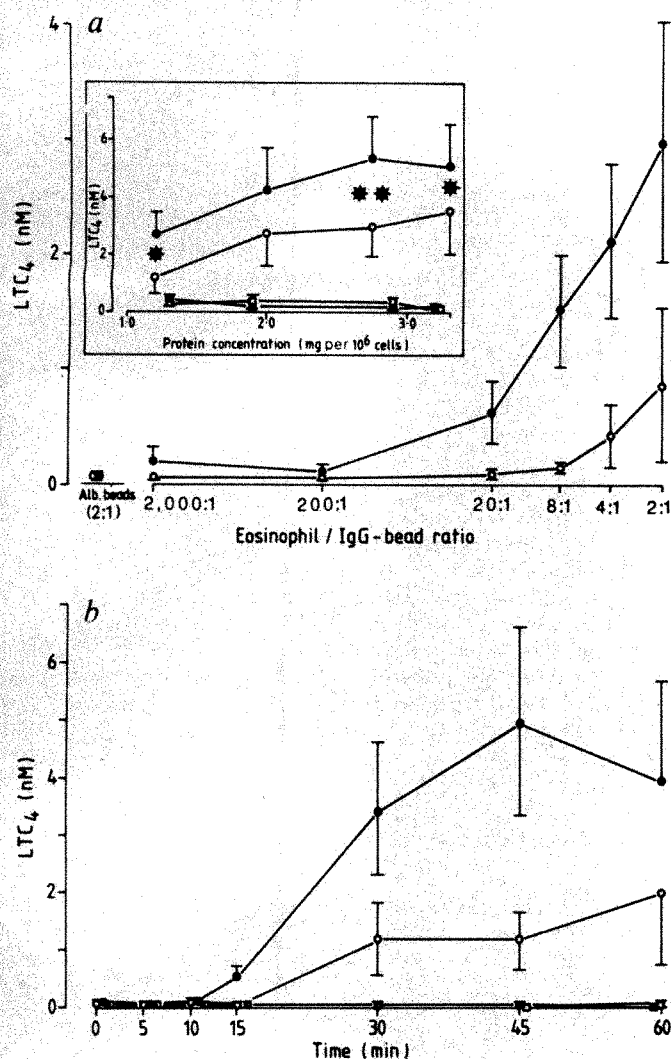
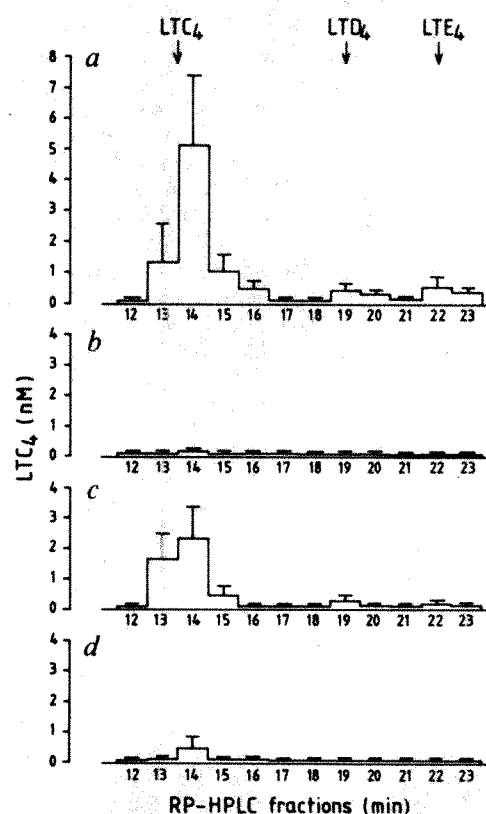


Fig. 2 Immunoreactive LTC₄ in the reverse-phase HPLC fractions of the eosinophil products from incubation of: *a*, low-density cells with IgG-coated beads; *b*, low-density cells with albumin-coated beads; *c*, normal-density cells with IgG-coated beads; *d*, normal-density cells with albumin-coated beads (mean $\pm$ s.e.m., $n=3$), were compared with the retention times of synthetic standards.

Methods. Immunoreactive LTC₄ in the reverse-phase (RP) HPLC fractions of supernatants from eosinophils incubated with IgG-coated beads. Eosinophils (10^6 ml^{-1}) were incubated with protein-coated beads as described in Fig. 1 legend. The leukotrienes in the supernatant were extracted in 80% methanol which was diluted to 10% with water and acidified to pH 4 before further extraction using a C18 Sep-Pak (Waters Associates)^{3,44}. The leukotrienes eluting in 100% methanol were evaporated to dryness, resuspended in running buffer (methanol/water/acetic acid, 70:30:0.1, adjusted to pH 5.4 with ammonia) and separated by RP-HPLC on a Nucleosil C18 column. The fractions were evaporated to dryness and resuspended before radioimmunoassay.

Fig. 1 *a*, Generation of LTC₄ by eosinophils in response to incubation with varying numbers of IgG-coated Sepharose beads with or without prior incubation with fMLP. Normal-density eosinophils (10^6 ml^{-1} , >90% purity) were incubated for 30 min with fMLP (10^{-8} M) (●) or buffer (○) followed by 30 min with varying numbers of IgG-coated beads (2.75 mg IgG per 5×10^5 beads) or albumin-coated beads (2.9 mg albumin per 5×10^5 beads) (mean $\pm$ s.e.m., $n=4$). Inset, effect of fMLP on LTC₄ generation by eosinophils incubated with IgG-coated beads. Normal-density cells (10^6 ml^{-1} , >90% purity) were incubated with fMLP or buffer for 30 min followed by 30 min with Sepharose beads ($5 \times 10^5 \text{ ml}^{-1}$) coated with varying doses of IgG or albumin. ●, fMLP + IgG-coated beads; ○, buffer + IgG-coated beads; ■, fMLP + albumin-coated beads; □, buffer + albumin-coated beads (mean $\pm$ s.e.m., $n=8$, * $P<0.05$, ** $P<0.02$). *b*, Time course of release of LTC₄ by eosinophils incubated with IgG-coated beads with enhancement by fMLP. Normal-density eosinophils (10^6 ml^{-1} , >90% purity) were incubated for 30 min with fMLP (10^{-8} M) or buffer, followed by addition of either IgG- (2.75 mg ml^{-1}) or albumin- (2.9 mg ml^{-1}) coated beads ($5 \times 10^5 \text{ ml}^{-1}$) and the incubation terminated after varying time intervals. Symbols, ●, fMLP + IgG-coated beads; ○, buffer and IgG-coated beads; ■, fMLP + albumin-coated beads; or □, buffer + albumin-coated beads (mean $\pm$ s.e.m., $n=4$).

Methods. Human eosinophils were purified on discontinuous metrizamide gradients⁴¹. Populations were identified on the basis of density, normal density (>1.12 g ml^{-1}) in fractions 4 and 5 and low-density cells (<1.12 g ml^{-1}) in fractions 2 and 3. The eosinophils (10^6 ml^{-1}) were incubated with either fMLP (10^{-8}) or buffer at 37 °C for 30 min. The cells were further incubated with human IgG- or human albumin-coated beads, which were left to sediment during the course of the incubation. Beads were prepared with cyanogen bromide-activated Sepharose 4B particles (Pharmacia). These particles were swollen in 1 mM HCl and washed with coupling buffer (0.2 M NaHCO₃ pH 8.7 containing 0.5 M NaCl). Aliquots (1 ml) of gel were incubated with 10, 7.5, 5 and 2.5 mg of human IgG or albumin (Sigma), dissolved in coupling buffer, for 2 h at room temperature with continuous mixing. The purity of the IgG preparation was determined by crossed immunoelectrophoresis (kindly performed by Dr Joan Longbottom). The preparation contained >98% IgG, with albumin and pre-albumin the principal contaminants. There was <0.001% IgE as assessed by a double-antibody radioimmunoassay⁴² (kindly performed by Dr T. G. Merrett). Any remaining reactive groups on the Sepharose were blocked by incubating particles with 1 M ethanolamine pH 8.0 for 2 h at room temperature with frequent mixing. The particles were washed alternately with coupling buffer and acetate buffer (0.1 M, pH 4.0, containing 0.5 M NaCl) to remove any noncovalently bound protein. This resulted in preparations containing 3.30, 2.75, 2.00 and 1.20 mg IgG per 5×10^5 beads, respectively, determined after alkaline digestion of the coupled beads. The incubation was terminated by centrifugation (8,000g for 1 min) and the supernatant stored at -80 °C before radioimmunoassay. Samples were assayed in duplicate using a specific sulphidopeptide leukotriene radioimmunoassay using antisera raised in rabbits which gave cross-reactivity between LTC₄, LTD₄ and LTE₄ of 100:70:8 (refs 3, 43, 44). Statistical analysis was performed using the Wilcoxon matched-pair signed rank test.



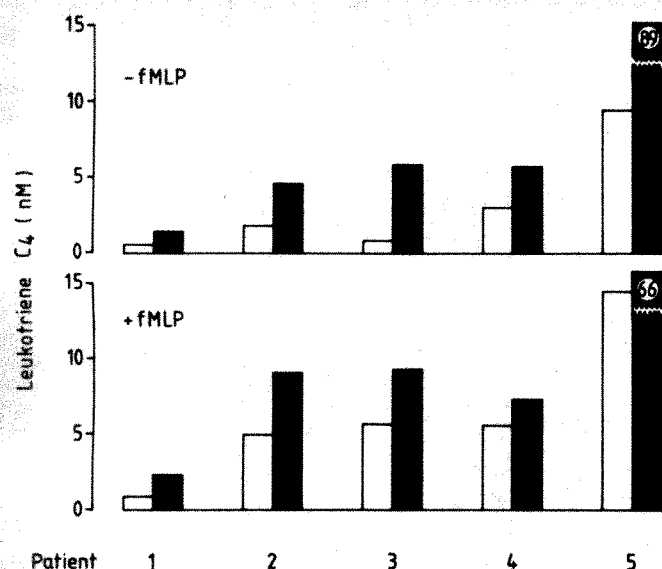


Fig. 3 LTC₄ generation by low-density and normal-density eosinophils incubated with IgG-coated beads with or without fMLP. The eosinophils were collected from four patients with hypereosinophilic syndrome and one with Churg-Strauss hyper-eosinophilia (patient 5). The cells were separated on metrizamide gradients into low density (metrizamide fractions 2 and 3, density <1.12 g ml⁻¹) and normal density (metrizamide fractions 4 and 5, density >1.12 g ml⁻¹). The cellular constituents of the low-density fraction from each of the patients were: patient 1, 67% eosinophils (E), 30% neutrophils (N), 30% mononuclear cells (M); patient 2, E, 38%; N, 54%; M, 8%; patient 3, E, 90%; N, 10%; M, 0%; patient 4, E, 54%; N, 46%; M, 0%; patient 5, E, 92%; N, 0%; M, 8%. The cellular constituents of fractions 4 and 5 were: patient 1, E, 95%; N, 5%; patient 2, E, 90%; N, 10%; patient 3, E, 94%; N, 6%; patient 4, E, 95%; N, 5%; patient 5, E, 91%; N, 9%. LTC₄ concentrations (mean $\pm$ s.e.m., $n=5$) were measured after incubating the low-density and normal-density eosinophils (10^6 ml⁻¹) for 30 min with fMLP (10^{-8} M) followed by IgG-coated (black columns) or albumin-coated (white columns) beads (5×10^5 ml⁻¹) at protein concentrations as shown (± 0.1 mg).

presence of albumin-coated beads was not associated with leukotriene generation. The leukotriene production by eosinophils was dependent on the number of beads present, with a maximal eosinophil-to-bead ratio of 2:1 (Fig. 1a).

The identities of the sulphidopeptide leukotrienes produced at 30 min by both normal- and low-density cells were confirmed by comparing immunoreactivity of the fractions with the retention times of synthetic markers using reverse-phase HPLC (Fig. 2). Both cell types generated LTC₄ following incubation with IgG-coated beads. A small amount of activity was present in fractions co-eluting with synthetic LTD₄, indicating partial degradation of LTC₄ to LTD₄. No sulphidopeptide leukotriene immunoreactivity was identified in the products of eosinophils incubated with albumin-coated beads.

LTC₄ generation in the presence of IgG-coated beads by low-density and normal-density eosinophils from five patients with eosinophilia was compared (Fig. 3). In each case the low-density cells generated greater amounts of LTC₄ than the normal-density cells from the same patients over a dose range of IgG. This increased production varied from 1.5 to 10 times that of the normal-density cells and was related to the proportion of eosinophils in metrizamide fractions 2 and 3. Neither normal- nor low-density cells generated LTC₄ when incubated with albumin-coated beads. Production of LTC₄ by low-density cells with IgG-coated beads (16 ng per 10^6 eosinophils) was less than that of normal-density cells treated with Ca ionophore A23187 (41 ng per 10^6 eosinophils)³; only a slight increase in LTC₄ generation was noted following preincubation of low-density cells with fMLP.

Confirmation that the LTC₄ originated from the normal- and low-density eosinophils was obtained by showing that

neutrophils failed to produce LTC₄, and that a mononuclear cell fraction, which also contained basophils (1.2%), generated <1.5 ng LTC₄ per 10^6 cells. We assumed cell viability in all experiments, as leukotrienes are not preformed and their synthesis following stimulation requires energy and is dependent on cell integrity.

The stimulus in this study was IgG (Fc), known to bind to eosinophils by specific Fc receptors¹¹⁻¹⁴. It has been shown previously that fMLP and other chemotactic factors enhance the expression of IgG (Fc) receptors on eosinophils, suggesting that the increased LTC₄ production observed here results from greater stimulus-response coupling^{35,36}. IgG aggregates alone did not consistently trigger LTC₄ generation by eosinophils. On the other hand, the beads provide a large surface similar to that which occurs in helminth cytotoxicity *in vitro*, in which eosinophil secretory activity is stimulated^{15,37}.

The demonstration of LTC₄ generation by activated human eosinophils in response to stimulation with IgG-coated beads may have important implications for human disease. Eosinophils involved in gastrointestinal helminthic infections³⁸ would have access to IgG-coated worms and the resultant leukotriene generation causing mucous hypersecretion and smooth muscle contraction might favour worm expulsion. Similarly, in allergies (where IgG antibodies accompany the IgE response³⁹) eosinophil-derived leukotrienes might amplify the mucus production, bronchospasm, hyperaemia and oedema associated with these conditions. Comparable amounts of LTC₄ have been found in body fluids during allergic reactions (for example, 20 pmol LTC₄ extracted from nasal secretions following allergen challenge⁴⁰).

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A group of genes controlling the spatial expression of the bithorax complex in *Drosophila*

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The morphological diversity among the body segments of *Drosophila*, as displayed in the pattern of the larval cuticle¹, arises during embryogenesis by the selective expression of homoeotic genes. For example, the bithorax complex (BX-C) confers abdominal development on the segments in the posterior half of the embryo which otherwise would develop into thoracic segments². Mutations in trans-regulatory genes such as *Polycomb* (*Pc*)³ or *extra sex combs* (*esc*)³ cause indiscriminate expression of the BX-C genes, which leads to all body segments being abdominal. I report here on mutations in four other trans-regulatory genes, members of a novel class of more than 20 genes controlling the spatial expression of BX-C genes. In contrast to *Pc* and *esc* mutations, only partial transformation into posterior abdominal development is observed in mutant embryos. However, embryos mutant for two or more of these genes show strong homoeotic transformation of all body segments similar to, or stronger than, that seen in *Pc* and *esc* embryos, indicating that these genes act synergistically in normal development to control the spatial expression of the BX-C genes.

Recent large-scale screens both for embryonic-lethal mutations affecting the pattern of the larval cuticle⁴⁻⁶ and for dominant 'extra sex combs' mutations (G.J., manuscript in preparation), yielded zygotic mutations in loci other than *Pc* which resemble weak *Pc* mutations in both their dominant adult and recessive embryonic phenotypes. These genes will be collectively referred to as the *Pc* group after its first known and most prominent member. Some of the mutations in the four *Pc* group loci described below were isolated for their embryonic head defect phenotypes, while most of them were identified on the basis of their dominant 'extra sex combs' trait in adult flies. The penetrance of this dominant phenotype depends on culture conditions and genetic background, but the recessive phenotype is embryonic lethality associated with an incomplete transformation of several body segments into more posterior abdominal segments. Three of these genes have not been identified previously, whereas one of them (*Polycomb-like Pcl*) appears to be identical to that described by Duncan⁷ (Table 1).

Mutations at the *Pcl* and *Sex comb on midleg* (*Scm*) loci cause posteriorly directed transformation of abdominal segments in the embryo. In the strongest alleles available, the first abdominal segment resembles the second abdominal segment and abdominal segments 2-7 are transformed into more posterior abdominal segments, while the head and thorax are normal (Fig. 1b). Weaker alleles affect only the more posterior segments, while the weakest *Pcl* allele is homozygous viable and transforms all the legs of adult flies to forelegs, including complete sex combs in males. This phenotype, which resembles the adult phenotype associated with mutations in *Pc* and other putative trans-regulatory genes^{3,7-10}, presumably results from the abnormal expression in the posterior leg disks of the *Sex combs reduced* gene, a member of the *Antennapedia* complex¹¹. Mutations at the *Posterior sex combs* (*Psc*) and *Additional sex combs* (*Asx*) loci represent a different class of phenotype in that they affect the entire body pattern of the embryo. The most prominent feature is a head defect while the posteriorly directed transformation is rather subtle in both head and thorax but clear in the abdomen (Fig. 1c). The incomplete homoeotic transformations associated with the *Pc* group mutations are apparently not the result of incomplete inactivation of these genes as the strongest alleles in *trans* to their corresponding deficiencies (*Pcl*, *Scm*) or the homozygous deficiencies (*Psc*, *Asx*) cause essentially the same phenotypes (Table 1). It should be noted that the lack-of-function phenotypes may be stronger than the amorphic phenotypes described here if these genes were also expressed during oogenesis.

Embryos which are homozygous mutant for any pair of the four *Pc* group loci show strong posteriorly directed transformation of the entire body pattern, resembling the amorphic *Pc* phenotype (Fig. 1d, e). The denticle bands of the trunk segments look very similar to one another and resemble the normal last abdominal denticle band. The head does not undergo involution and two abdominal denticle bands appear there, one ventrally in the gnathal region between the prothorax and the pharynx, and another on the dorsal surface of the head. Although *Pcl* and *Scm* individually appear to affect only the abdomen, the strong transformation seen in double mutant embryos (Fig. 1d) indicates that the *Pcl* and *Scm* gene functions are required for normal development not only in the posterior but also in the anterior half of the embryo.

The embryonic phenotype of the triple mutant *Psc Asx Pcl* is characterized by a tandem array of posterior abdominal segments including up to four abdominal denticle bands in the head region (Fig. 2a). If this transformation were caused by indiscriminate expression of BX-C genes, it should be suppressed in embryos which also lack the BX-C genes as is the

Table 1 Genetic and phenotypic features of *Pc* group genes

Locus	Map position	Cytology*	No. of alleles†	Embryonic phenotype‡
<i>Additional sex combs</i> (<i>Asx</i>)	2-72	51AB	6 (3)	H,T to (A); A1-7 to Ap
<i>Polycomb-like</i> (<i>Pcl</i>)§	2-83	55AF	8 (5)	A1 to (A2); A2-7 to Ap
<i>Posterior sex combs</i> (<i>Psc</i>)	2-67	49EF	1	H,T to (A); A1-7 to Ap
<i>Sex comb on midleg</i> (<i>Scm</i>)	3-49	85EF	4 (2)	A1 to (A2); A2-7 to Ap

* Assignments to polytene chromosome regions are based on the inclusion of the locus in a deficiency: *Asx* in *Df(2)L⁺48*; *Pcl* in *Df(22)PC4*; *Psc* in *Df(2R)vg^D* and *Df(2R)vg^B* but not in *Df(2R)vg^C* (ref. 4); *Scm* in *Df(3R)by¹⁰* and *Df(3R)by⁶²* (ref. 13) but in neither *Df(3R)roe^{XM4}* nor the deficiency segregated by T(2;3)Ctx (ref. 5).

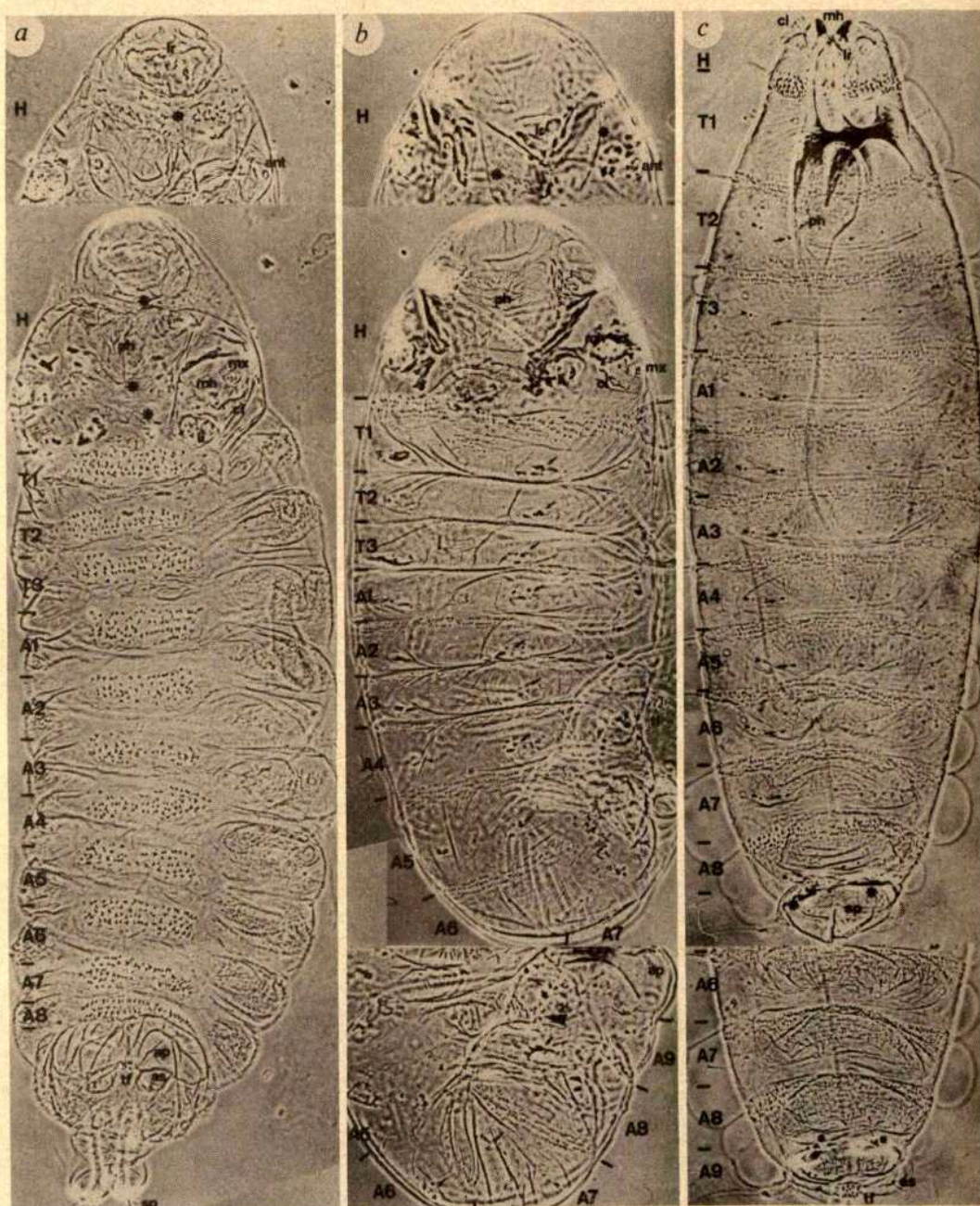
† Two mutants, one *Asx* allele (IIF51) and the *Psc* allele (IIN48), were isolated as embryonic lethal mutations causing head defects⁴. Two *Pcl* alleles (E33 and E90) were isolated as dominant extra sex combs mutations by G. Struhl. One *Asx* allele (P2) was isolated as a dominant enhancer of *Pc* by Dura *et al.*⁸. All other mutants were isolated on the basis of their dominant 'extra sex combs' phenotype in the F₁ male progeny of either ethyl methane sulphonate-treated (50 mM, 24 h) or X-irradiated (4,000 R, 100 kV, 10 mA, 1 mm Al filter, 350 R min⁻¹, Philips MG102) males of one of the following genotypes: *b pr cn sca*, *b pr cn wtx bw*, *st e*, *cp in ri p^P* (ref. 14). Numbers of weak alleles are given in parenthesis. Classification of alleles as either strong or weak is based on the strength of the embryonic phenotype which, however, varies depending on the genetic background. So weak and strong alleles at any of the four loci may show very similar phenotypes, except *Pcl^{XT56}*, which is homozygous viable.

‡ Amorphic or probably amorphic phenotypes: H,T to (A), abdominal denticles in head and thorax; A1 to (A2), incomplete transformation of abdominal segment 1 into segment 2; A1-7 or A2-7 to Ap, abdominal segments 1-7 or 2-7 transformed to more posterior segments, often in a graded fashion with segments 5-7 closely resembling A8.

§ *Pcl* is probably identical to the *Pcl* locus described by Duncan⁷ as judged by map position, cytology and similarity of phenotype.

|| Chao-ting Wu (personal communication) has found that alleles of *Suppressor of zeste-2* do not complement the lethality of *Psc^{IIN48}*. However, the three alleles 1, 4 and 5 of *Su(z)2* do not share the embryonic phenotype of *Psc^{IIN48}* and *vg^D* (unpublished data) so the allelic relationship between *Psc* and *Su(z)2* should be considered unclear.

Fig. 2 Comparison between *Psc Asx Pcl* (a), *Psc Asx Pcl;P9* (b) and *P9* (c) embryos. *P9* deletes the entire BX-C^{2,16}, causing thoracic transformation of abdominal denticle bands and resulting in the presence of chitinous structures posterior to transformed segment A8^{2,17}. a, Extreme transformation of all body segments towards A8. Abdominal denticle bands appear in the head region at four different levels, one on the dorsal surface, one anterior and two posterior to the floor of the pharynx (asterisks). Head involution is blocked so the remnants of head structures remain on the outside. b, Suppression of abdominal features. Instead, mesothoracic denticles appear on the dorsal surface of the head (asterisks) and in trunk segments T1 to A8. Note the supernumerary A9 which bears prothoracic denticles (arrowhead) and the presence of chitinous plates in most body segments (arrows) which indicate transformation towards head structures (compare posterior end of *P9* embryo). c, Segments T2 to A7 show mesothoracic denticle bands, while the denticle band of A8 appears to be intermediate between normal T1 and T2. Note the presence of Keilin's organs (arrows) and black dots (arrowheads) in all trunk segments and the presence of chitinous plates posterior to A8 (asterisks). Dorsally, the segments T2 to A8 appear to be composed of anterior T2 and posterior T1, and a ninth abdominal segment is recognizable between the chitinous plates (asterisks) and the tuft. ap, Anal plate; as, anal sense organ; mx, maxillary sense organ; sp, posterior spiracles; tf, tuft. Other abbreviations as for Fig. 1.



expressed *Pc* group genes suggests a surprising complexity of the *trans*-regulatory system that controls the spatial expression of homeotic genes. It is conceivable that such a complex system is better buffered against perturbation and thus better adapted in evolutionary terms than would be a spatial control by only one or a few *trans*-regulatory genes. If one were to view segment diversity as the result of evolutionary modification of a single repeated pattern¹², slight genetic changes in *trans*-regulatory genes controlling the spatial expression of homeotic genes might gradually have built up the necessary genetic isolation between segments to ensure largely independent evolution of segment-specific features.

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Local degradation of fibronectin at sites of expression of the transforming gene product pp60^{src}

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Local degradation of extracellular fibronectin, a major extracellular adhesive protein, is believed to play an important part in the migration of cells through the extracellular matrix during tumour invasion, morphogenetic movement and trophoblast implantation¹⁻⁵. Fibronectin is lost from the cell surface after transformation with Rous sarcoma virus (RSV)⁶. By using fluorescent and radiolabelled probes covalently coupled to the surface of substrata, we have recently identified a proteolytic activity that is expressed in RSV-transformed cells and is involved in the local degradation of fibronectin at cell-substratum contact sites⁷. Here, we extend the relevance of these findings and gain some insight into the cellular functions of pp60^{src}, the transforming gene product of RSV. We show that newly expressed viral pp60^{src} is localized at the cytoplasmic surface of the cell membrane, corresponding to the cell contact sites where degradation of extracellular fibronectin occurs.

Localized fibronectin degradation was detected in cells cultured on fixed gelatin films coupled with rhodamine- or radio-labelled fibronectin in the presence of 10% fetal bovine serum. The appearance of black spots on the uniformly fluorescent surface of the gelatin film indicated sites of local fibronectin degradation. The resulting release of labelled fibronectin into the medium provided a measure of the cleavage of the polypep-

tides by the cells⁷. Because there was an induction of rosette contacts on RSV transformation of cells concomitant with a redistribution of α -actinin and vinculin to rosette contacts⁷⁻¹¹, we used monoclonal antibody N.353 directed against α -actinin for immunolabelling of the rosettes in the transformed cells in the above preparation, in order to determine whether the local degradation of fibronectin occurs at rosettes.

The system chosen for the study of cell transformation consisted of primary cultures of chicken embryo fibroblasts (CEF) infected with a temperature-sensitive mutant (ts68) of RSV¹². Infected cells exhibited the transformed phenotype when grown at the permissive temperature (37 °C) after a shift from the non-permissive temperature (41 °C). ts68-CEF grown at the permissive temperature for various times therefore allow a direct analysis of the transformed phenotype. Figure 1 shows that within 1 h after the temperature shift (Fig. 1a-c), a few black spots appeared on the rhodamine-fibronectin substrata (Fig. 1b) and these coincided with the immunoreactive α -actinin-rich rosette contacts of transformed cells (Fig. 1c). However, within 6 h after the shift (Fig. 1d-f), the cells became rounded, a morphological change that was accompanied by increasing numbers of degraded fibronectin spots (Fig. 1e) coinciding with α -actinin-rich rosette contacts (Fig. 1f). As shown previously in RSV-transformed cells⁷, the degraded spots appear at the rosettes (Fig. 1, open arrows), but never at the focal adhesions (Fig. 1, fluorescent streaks). In addition, the pattern of degraded spots is distinct from that of fibronectin displacement from non-coupled substrata exhibited by normal or transformed cells⁷. Furthermore, the initial release of radioactivity from the fibronectin substrata was detectable by 6 h after the temperature shift in ts68-CEF cultures (result not shown). The released material was characterized as peptides of relative molecular mass (M_r) < 30,000. Thus, localized degradation of extracellular fibronectin occurs at sites of rosette contacts of RSV-transformed cells.

Previous efforts to localize pp60^{src} all involved the use of polyclonal antisera^{9,10,13-15}. Because these antisera react with both cellular and newly expressed viral pp60^{src}, we examined

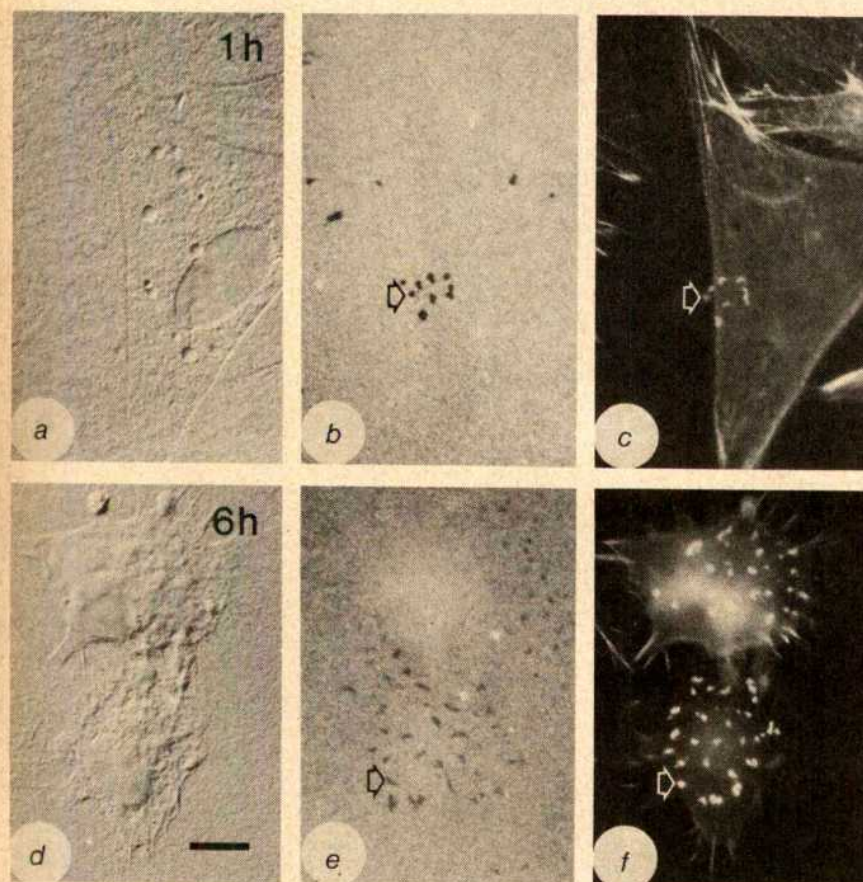
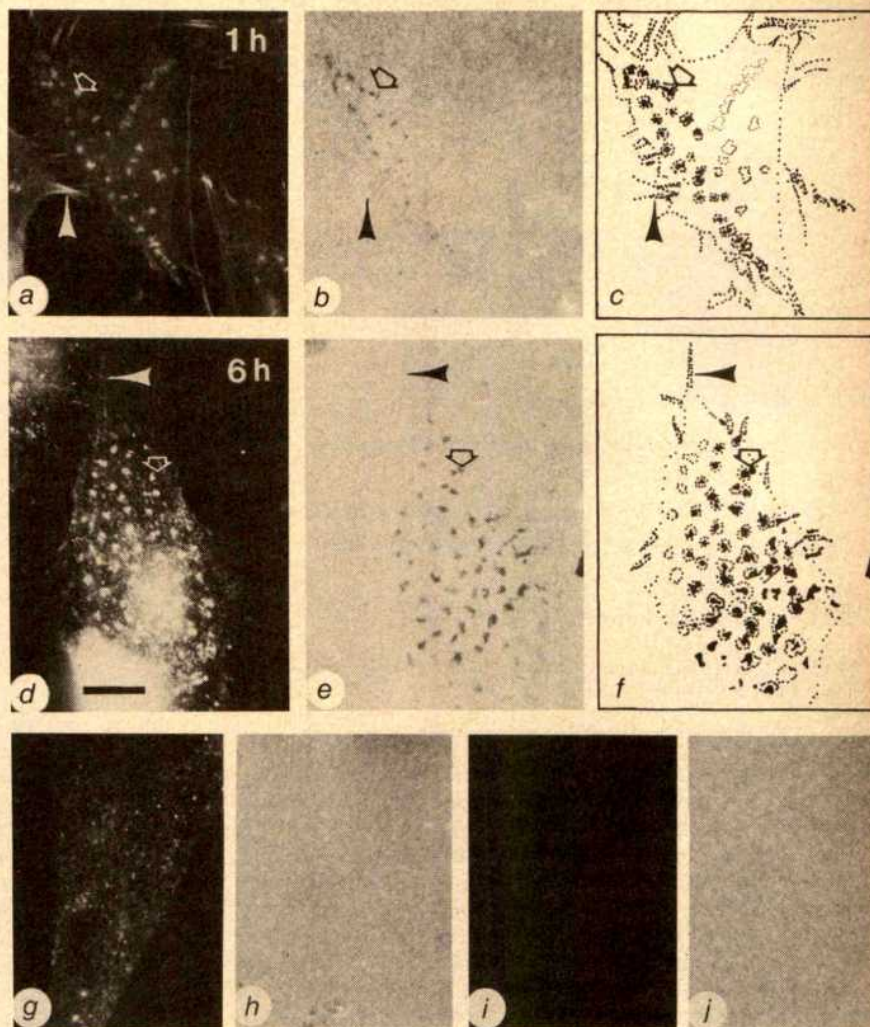


Fig. 1 Demonstration of the time course of expression of a transformation-associated protease that degrades fibronectin at rosette contact sites. ts68-infected CEF were grown at the permissive temperature (37 °C) for 1 h (a-c) and 6 h (d-f) after a temperature shift from 41 °C. a, d, Differential interference contrast images of the cells. b, e, Fluorescent images of rhodamine-labelled fibronectin substrata showing fields of the same cells as in a and d, respectively. Black spots (open arrowheads) represent areas where fibronectin has been locally degraded. c, f, Immunofluorescence images of the same cells as in a and d, respectively, using indirect monoclonal antibody labelling for intracellular α -actinin. α -Actinin is redistributed on transformation to rosette sites (open arrowheads) which coincide with the black spots in b and e.

Methods. The ts68-infected CEF were cultured in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum on rhodamine conjugates of fibronectin that were covalently linked to the surface of a fixed gelatin film¹⁷. The cultures were then shifted to the permissive temperature (37 °C) for various periods of time before fixation and permeabilizing with 0.5% Triton X-100 for antibody labelling. The fixed cells were labelled indirectly, first with mouse monoclonal antibody N.353 directed against α -actinin (Amersham Corp., Arlington Heights, Illinois), then with fluorescein conjugates of goat antibody to mouse IgG and IgM. Labelled cells were observed with a Zeiss Photomicroscope III equipped with epifluorescence and a Planapo 63/1.4 objective. Scale bar, 10 μ m.

Fig. 2 Coincidental localization of viral pp60^{src} and fibronectin degradation at sites of rosette contacts. Normal CEF (*i, j*) and ts68-infected cells were grown either at the non-permissive temperature (41 °C) (*g, h*), or at the permissive temperature (37 °C) for 1 h (*a-c*) and 6 h (*d-f*) after a temperature shift from 41 °C. The ts68 RSV-transformed cells were cultured on rhodamine-fibronectin substrata according to the procedure described in Fig. 1 legend, except that fixed cells were indirectly labelled with EB7 directed against an epitope of viral pp60^{src}. *a, d*, Immunofluorescence images of transformed cells labelled with EB7 for localization of intracellular viral pp60^{src}. Open arrowheads indicate rosette contacts that appear after cell transformation. White arrowheads point to focal adhesions, which are commonly found in both normal and transformed cells. *b, e*, The rhodamine-fibronectin substrata under the cells shown in *a* and *d*, respectively. Black spots represent sites of local fibronectin degradation, and are present at areas corresponding to rosette contacts (open arrowheads), but absent at focal adhesion (solid arrowheads) and areas free of cell contact. *c, f*, Overlay tracing of pp60^{src} localization in the cells of *a* and *d* (dotted outline) and of degraded fibronectin spots on substrata of the same fields (solid areas). *g, h*, Viral pp60^{src} labelling of ts68-CEF grown at 41 °C (*g*) and its underlying labelled fibronectin substrata (*h*). *i, j*, Control for viral pp60^{src} labelling in normal CEF (*i*) and the corresponding labelled fibronectin substrata (*j*). The same exposure time was used for photographing the viral pp60^{src} labellings in all preparations. Scale bar, 10 µm.



the intracellular localization of viral pp60^{src} by using monoclonal antibody EB7, which recognized an epitope in the amino-terminal region (residues 1-82) of the viral pp60^{src}, but did not efficiently recognize determinants on the cellular pp60^{src} homologue^{16,17}. To determine whether local removal of fibronectin also occurs at the sites of expression of viral pp60^{src} on RSV transformation, ts68-CEF were grown on rhodamine-fibronectin substrata at the non-permissive temperature (41 °C), shifted to 37 °C for various periods of time as described above, then immunolabelled with EB7 to compare the time-course correlation between viral pp60^{src} localization and the sites of fibronectin degradation. In normal CEF grown at 41 °C or 37 °C, we observed neither viral pp60^{src} labelling nor degraded spots (Fig. 2*i, j*). In ts68-CEF grown at 41 °C, viral pp60^{src} labelling was diffuse in the cytoplasm, but no local fibronectin degradation was detected (Fig. 2*g, h*). Within 1 h after the temperature shift to 37 °C, viral pp60^{src} was localized at both focal adhesions and rosette contacts (Fig. 2*a, c*), but mostly within rosettes at later times (Fig. 2*d, f*). Concomitantly, local fibronectin degradation was found mostly at rosettes, but not at focal adhesions (Fig. 2*b, c, e, f*). As shown in Fig. 1, however, there is not a perfect one-to-one correspondence between pp60^{src} and spots of fibronectin degradation, indicating that some of the rosettes either show little proteolytic activity or have become detached from the original sites. These results suggest a temporal and spatial relationship between rosette formation, local fibronectin degradation and viral pp60^{src} expression in RSV-transformed cells. It appears that viral pp60^{src} is produced in cells at the non-permissive temperature and becomes accumulated in functional sites at rosettes after the shift to the permissive temperature.

Functional expression of pp60^{src} is required for manifestation of the oncogenic events which are initiated on RSV infection^{12,18}.

Several studies have shown that viral pp60^{src} has the following characteristics: (1) it has an intrinsic tyrosine-specific phosphotransferase activity^{19,20}; (2) it is localized on the cytoplasmic face of the plasma membrane²¹; (3) its target proteins include the adhesion plaque component vinculin¹³; and (4) it has a cellular homologue²², which has been identified recently. The common localization for viral pp60^{src} and fibronectin degradation at rosette contacts demonstrated here suggests membrane-associated sites of action for both pp60^{src} and the extracellular protease. The pp60^{src} kinase may exert its primary effects on the plasma membrane at the rosettes through phosphorylation of integral membrane components²³ or of cytoplasmic vinculin^{9,13}. Such modification may directly or indirectly induce protease activity on the rosettes and thereby cause the local dissolution of fibronectin from the extracellular matrix. Such an effect could in turn have an important role in the local decrease in cell-substratum adhesion, in the rounding-up of the cell, and in the disorganization of the stress fibres that accompanies transformation of fibroblasts by RSV.

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Overproduction of p53 antigen makes established cells highly tumorigenic

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The p53 cellular tumour antigen, long known to be overproduced in a variety of neoplastically transformed cells^{1,2}, was recently shown to be directly involved in transformation. Thus, p53 can complement activated Ha-ras in transforming secondary rat embryo fibroblasts into grossly altered, tumorigenic cells^{3,4}. Moreover, p53 can also be shown to possess immortalizing activity⁵. Our previous results indicated, however, that the contribution of p53 to the transformation was not synonymous with immortalization, suggesting that the two activities of the protein are probably separable⁶. We demonstrate here that this is indeed the case, as overproduction of p53 in an established cell line, while not causing gross morphological changes, endows these cells with an overt tumorigenic potential. Furthermore, the tumorigenic efficiency of such cell lines may be correlated with the extent of p53 overproduction.

Co-transfection of rat embryo fibroblasts with a combination of activated Ha-ras and transcriptionally-potentiated p53 effectively generates morphologically transformed cells, capable of overgrowing the growth-arrested monolayer of normal cells^{3,4}. However, only a small fraction of such transformed cells are capable of continuous growth³. It was therefore proposed that

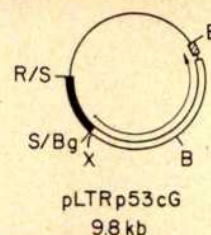
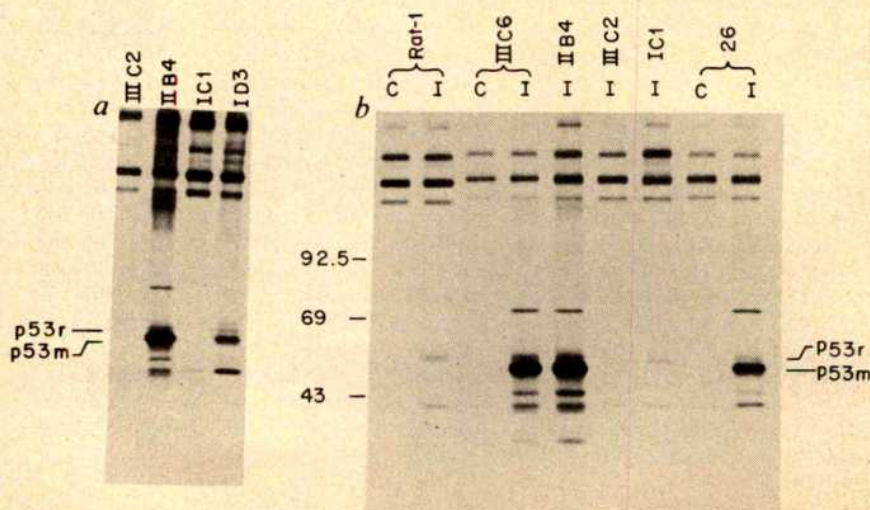


Fig. 1 Map of plasmid pLTRp53cG. To generate this construct, the DNA of plasmid pSVp53cG3 (ref. 12) was cleaved with *EcoRI* and *BglII* to remove simian virus 40 (SV40) sequences and then treated with the Klenow fragment of *Escherichia coli* DNA polymerase I to generate blunt ends. In parallel, a cloned proviral Harvey murine sarcoma virus (clone H-1; ref. 13), was cleaved with *SmaI*. The 1.3-kilobase fragment containing the retroviral 5'LTR (long terminal repeat) was isolated and introduced into the above blunt-ended vector. The resultant plasmid, pLTRp53cG, contains the LTR-derived enhancer, promoter and cap sites¹⁴, followed by a chimera of p53-specific DNA and genomic DNA¹². Solid bar, LTR sequences and adjacent 5' flanking cellular sequences; open bar, p53-specific sequences; thin line, pBR322 DNA; hatched bar, SV40 DNA. B, *BamHI*; Bg, *BglII*; R, *EcoRI*; S, *SmaI*; X, *XhoI*. Arrow indicates expected direction of transcription.

although nuclear oncogene products may have immortalizing potential, as was later in fact demonstrated for p53⁵, their ability to participate in neoplastic transformation may reflect an entirely different facet of their activity³. If this were the case, one might anticipate that certain types of established, non-transformed cells could be converted into more neoplastic ones by the mere overproduction of p53.

To test this assumption, we attempted to generate cell lines which over-produced the p53 antigen. Rat-1 cells⁶ were co-transfected with a mouse p53-specific expression vector together with a dominant selectable marker, ptkgpt (given by M. Shani). In the present study, we used a construct containing a chimera of p53 complementary DNA and genomic DNA (pLTRp53cG; Fig. 1). Rat rather than mouse cells were chosen because the p53 proteins of the two species can be easily differentiated with the aid of specific monoclonal antibodies, thus ruling out the possibility of endogenous rat p53 being nonspecifically induced by irrelevant parts of the transfecting plasmids. Mycophenolic-acid-resistant colonies were isolated⁷, expanded and analysed for the expression of mouse p53. Of 24 clones tested, most expressed little or no detectable murine p53. Only two lines displayed a very prominent overproduction of mouse p53, while two others made more moderate amounts of this protein. Figure 2 depicts the analysis of several such cell lines, using either a

Fig. 2 Detection of p53 in Rat-1-derived cell lines. Cells were grown in 60-mm dishes and labelled with 30 μ Ci ³⁵S-methionine per dish for 4 h. Proteins were extracted, immunoprecipitated and analysed by SDS-polyacrylamide gel electrophoresis as described previously⁸. Immunoprecipitation was with either monoclonal antibody RA3-2C2, specific for mouse p53¹⁵ (a), control serum (b, lanes labelled C) or monoclonal antibody pAb421, (L21)¹⁶, recognizing both mouse and rat p53¹⁵ (b, lanes I). Rat-1 is the parental cell line used for transfections; clone 26 is derived from rat embryo fibroblasts transformed by p53 plus Ha-ras³; all other cell lines were generated by co-transfection of Rat-1 cells with a p53-specific expression plasmid and ptkgpt. Each lane represents the polypeptides precipitated from 1.8×10^6 trichloroacetic acid-insoluble c.p.m. p53r and p53m denote the positions of rat and mouse p53, respectively. Numbers to the left of b refer to the relative molecular mass ($\times 10^{-3}$) of co-electrophoresed markers.



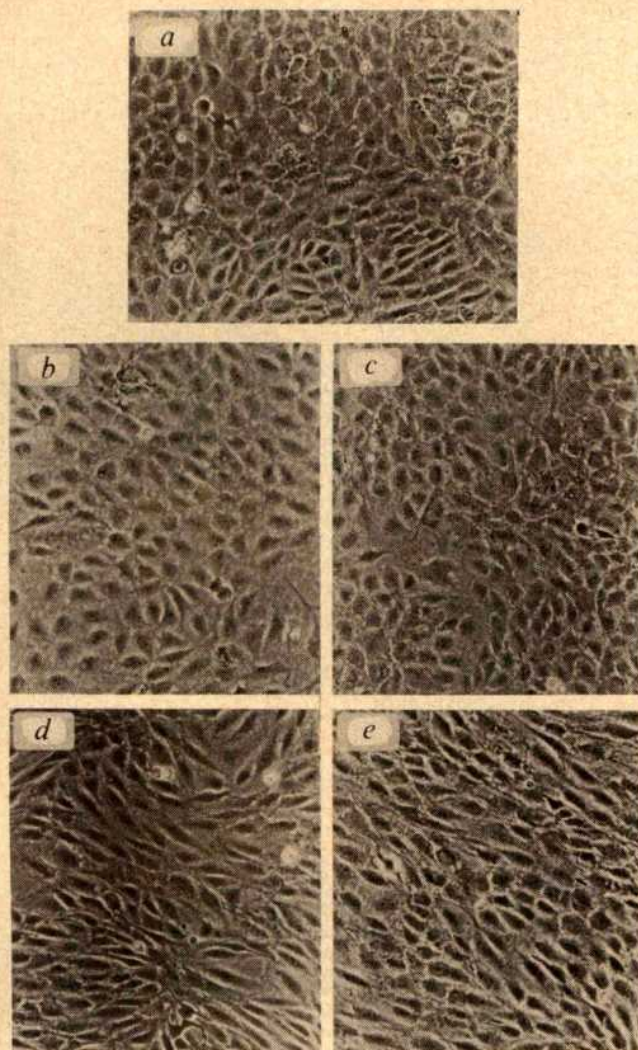


Fig. 3 Morphology of Rat-1-derived cells (phase-contrast photomicrographs). *a*, Parental Rat-1 cells; *b*, IB2; *c*, IIIC6; *d*, IIB4; *e*, IIB4T, a cell line established from a tumour induced in a nude mouse by injection of IIB4 cells. $\times 83$.

mouse p53-specific antibody (Fig. 2) or one reactive with both the mouse and rat species (*b*). In two lines (IIB4 and IIIC6), the production of mouse p53 well exceeds that of clone 26, a tumorigenic cell line derived by co-transfection of rat embryo fibroblasts with p53 plus Ha-ras (ref. 3 and D.E. *et al.* in preparation).

Unlike the case for p53 plus Ha-ras co-transformants, the p53-overproducing Rat-1 cells exhibited no dramatic morphological changes (Fig. 3). Some cell lines, such as IIB4, possessed a slightly altered shape while others, such as IIIC6, were practically indistinguishable from the parental Rat-1.

Overproduction of p53 can sometimes be due to post-translational events^{8,9}. Hence, it was of interest to determine whether the augmented levels of the protein in these cell lines reflected, as expected, the presence of larger amounts of the corresponding messenger RNA. Total cytoplasmic RNA was prepared from several lines and analysed directly by electrophoresis on a denaturing gel and hybridization with a mouse p53 cDNA probe. Both IIB4 and IIIC6, marked producers of the protein, contain conspicuous quantities of p53 mRNA (Fig. 4).

To test whether p53 overproduction could alter the biological properties of established cells, representative cell lines were injected into nude mice and monitored for the appearance of tumours. The results, summarized in Table 1, clearly demonstrate that both p53-overexpressing lines are highly tumorigenic, clone IIB4 being more efficient than IIIC6. Since IIB4 appears



Fig. 4 Detection of p53-specific transcripts in various cell lines and in a tumour. Total cytoplasmic RNA was extracted from several cell lines (first five lanes) by the Nonidet-P40 method¹⁷. In parallel, total cellular RNA was prepared from a tumour induced in a nude mouse by the injection of IIB4 cells (lane IIB4T) using the guanidine thiocyanate procedure¹⁸. RNA (8 μ g) was electrophoresed through a formaldehyde agarose gel¹⁹, blotted onto nitrocellulose²⁰ and hybridized with the 950-base pair p53-specific cDNA insert of clone pp53-176 (ref. 21) as described previously²².

Table 1 Tumorigenicity of Rat-1-derived cell lines in nude mice

Cell line	Mouse p53	2 Weeks		4 Weeks		8 Weeks	
		Score	Size	Score	Size	Score	Size
Rat-1	—	0/4	—	0/4	—	1/4	4
IIIC2	—	0/2	—	0/2	—	ND	
IIIC6	++	0/5	—	4/5	3–5	5/5	3–12
IIB4	+++	8/11	4–7	11/11	8–20		

CD1 *nu/nu* mice were injected subcutaneously with 5×10^6 cells. The numbers and sizes of resultant tumours were monitored for 8 weeks after injection. Score values indicate the number of tumour-bearing mice over the total number of animals injected with the particular cell line. Also shown is the range of sizes (in mm) of the observed tumours. ND, not determined.

to make more p53 than IIIC6, as determined by radiolabelling (Fig. 2), it is possible that the extent of p53-overexpression may determine the tumorigenic potency of these cells. It should be noted that a small tumour became visible after 8 weeks in one mouse injected with parental Rat-1 cells; this is in accord with the previously described ability of these cells to cause tumours with a long latency⁶. Thus, p53 may be markedly enhancing a latent tumorigenic potential of established cells rather than conferring such a potential *de novo*.

The rapid rate of tumour appearance following injection of IIB4 cells suggests strongly that their tumorigenicity is a property of the whole population rather than of a minor subset of different, fully transformed cells. Nevertheless, it was desirable to determine whether the cells comprising the tumour were not fundamentally different from those initially injected. To that end, we first determined the level of p53 mRNA in the tumour. The result (Fig. 4, lane IIB4T) clearly indicates that the tumour still makes very large quantities of p53 mRNA of the expected size; there is also a band of high relative molecular mass, not seen in tissue-cultured IIB4 cells. As the tumour RNA was extracted by a procedure which still retains the nuclear fraction, this higher band probably represents a precursor accumulating to high levels in the nuclei.

To further characterize such tumours, we excised one of them and placed it in tissue culture. The resultant cell line, IIB4T, had a morphology completely indistinguishable from that of the

parental IIB4 line (Fig. 3). This is in marked contrast to the results of a similar analysis on tumorigenic lines derived from p53 plus Ha-ras transformants (D.E. *et al.*, in preparation). Finally, protein analysis revealed that IIB4T possess the same level of p53 as IIB4 (data not shown), suggesting that the cells constituting the tumour are identical to those initially injected.

The data presented here argue strongly that the mere overproduction of p53 is sufficient to cause established cell lines to become tumorigenic. While this work was in progress, essentially similar findings were reported for the *myc* oncogene¹⁰, which also has several other properties in common with p53 (refs 3, 4, 11). As the immortalizing capacity of these nuclear oncogene products is irrelevant for established cell lines, one must conclude that both genes possess additional biological activities which are directly involved in tumour formation, while having no discernible effect on the apparently normal morphology of the cells. Whether these additional activities are the same for p53 and *myc* remains to be determined.

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Human N-myc gene contributes to neoplastic transformation of mammalian cells in culture

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Proto-oncogenes represent a group of eukaryotic genes whose activated forms are implicated in the development of cancer (for reviews, see refs 1-3). We have recently identified a human gene, *N-myc*, that is distantly related to the proto-oncogene *c-myc*⁴. *N-myc* is expressed at abnormally high levels consequent to amplification in numerous human neuroblastoma cell lines and metastatic neuroblastoma tumours^{5,6}. In addition, enhanced expression of *N-myc*, often a result of amplification, has been found in retinoblastoma cell lines and tumours (refs 5, 7 and M.S., unpublished data) and in cell lines derived from small-cell car-

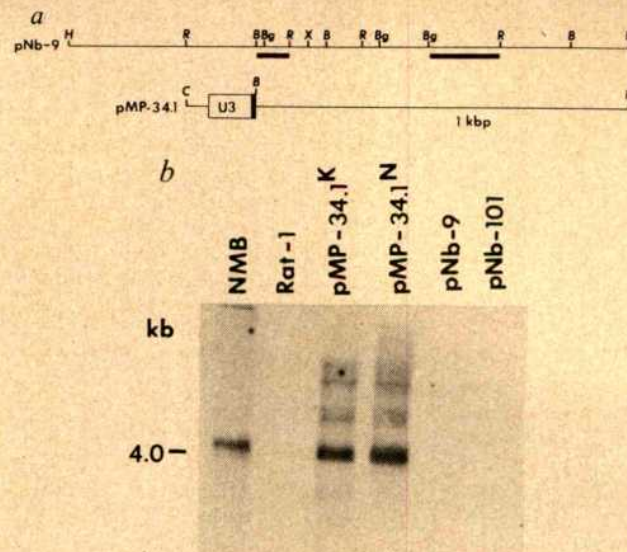


Fig. 1 Molecular clones of *N-myc*. *a*, Partial restriction endonuclease maps. The clones pNb-9 and pMP-34.1 were used for transfections. H, *Hind*III; R, *Eco*RI; B, *Bam*HI; Bg, *Bgl*II; X, *Xho*I. Heavy lines beneath the map of pNb-9 indicate the domains within which the 5' and 3' ends of *N-myc* must lie. Transcriptional orientation is from left to right. The U3 region in clone pMP-34.1 is derived from a M-MuLV LTR. The *Bam*HI site in the LTR used for attachment to *N-myc* is normally not present, but is the result of insertion of a polylinker in the natural *Kpn*I site within the R region of the Moloney murine leukaemia virus LTR. *b*, Expression of *N-myc* clones in Rat-1 cells. *N-myc* constructs were co-transfected together with a gene conferring resistance against the drug neomycin (molar ratio ~10:1) according to routine procedures¹⁰, and resistant cells were selected using the drug G418 (Gibco; 800 μ g ml⁻¹ crude). Cells were then grown in mass culture, polyadenylated RNA was isolated from a cytoplasmic fraction, and 5 μ g per lane was separated on a 1% agarose gel in the presence of 2.2 M formaldehyde. The RNA was transferred to nitrocellulose paper which was then probed with ³²P-labelled insert of plasmid clone pNb-1 (ref. 4). pNb-101 represents a cosmid clone containing a ~35 kb insert encompassing *N-myc*. NMB represents Kelly, a human neuroblastoma cell line.

cinomas of the lung⁸. Here, we show that enhanced expression of *N-myc* subsequent to co-transfections of an *N-myc* expression vector and the mutant *c-Ha-ras-1*(EJ) (from the human bladder carcinoma cell line EJ) is a factor in tumorigenic conversion of secondary rat embryo cells. The transformed cells elicit tumours in athymic mice and isogeneic rats. The ability of *N-myc* to contribute to neoplastic transformation of cultured mammalian cells raises the possibility that enhanced expression consequent to amplification of *N-myc* may be a factor in the aetiology of human neuroblastoma.

To design a suitable *N-myc* expression vector, we have generated a preliminary definition of the boundaries of *N-myc* in human DNA. We found that *N-myc* is localized within an ~16.0-kilobase (kb) *Hind*III fragment (Fig. 1*a*; clone pNb-9) (M.S. *et al.*, manuscript in preparation). The 5' end of *N-myc* appears to reside within a 1.0-kb *Bam*HI/*Eco*RI fragment; the 3' end maps within a 2.0-kb *Bgl*II/*Eco*RI fragment in close proximity to the *Eco*RI restriction endonuclease site. A DNA fragment containing the U3 region of a long terminal repeat (LTR; clone pMP-1) of Moloney murine leukaemia virus (M-MuLV) was covalently linked to *N-myc* derived from the Kelly neuroblastoma cell line and from a normal individual, to generate clones pMP-34.1^K and pMP-34.1^N, respectively. Rat-1 cells, into which either one of these clones was introduced by transfection of DNA co-precipitated with calcium phosphate⁹, produced high levels of an ~4.0-kb *N-myc* transcript, as do human neuroblastoma cells containing amplified *N-myc* (Fig. 1*b*). *N-myc* messenger RNA was not detected in Rat-1 cells into which

Table 1 Transformation of rat embryo cells

Gene transfected	Clone*	No. of foci†
LTR/N-myc (Kelly) + c-Ha-ras-1 (EJ)	pMP-34.1 ^K + pEJ(6.6)	30-40
LTR/N-myc(normal) + c-Ha-ras-1 (EJ)	pMP-34.1 ^N + pEJ(6.6)	30-40
LTR/N-myc(Kelly; mutant) + c-Ha-ras-1(EJ)	pMP-34.1 ^K ΔXho + pEJ(6.6)	0
N-myc (Kelly) + c-Ha-ras-1 (EJ)	pNb-9 ^K + pEJ(6.6)	0
N-myc (normal) + c-Ha-ras-1 (EJ)	pNb-9 ^N + pEJ(6.6)	0
N-myc (Kelly) + LTR	pNb-9 ^K + pMP-1	0
N-myc (normal) + LTR	pNb-9 ^N + pMP-1	0
c-Ha-ras-1 (EJ) + LTR	pEJ(6.6) + pMP-1	0
LTR/N-myc (Kelly)	pMP 34.1 ^K	0
LTR/N-myc (normal)	pMP 34.1 ^N	0
c-Ha-ras-1	pEJ(6.6)	0
LTR	pMP-1	0

Primary cultures of rat embryo cells (REC) were prepared from 12-14-day-old Fisher rat embryos. After 2-4 days of growth in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS; Hyclone) and antibiotics, the cells were seeded at a density of 1×10^6 cells per 100-mm dish. Transfections were performed using 75 μ g of carrier DNA from salmon sperm plus roughly equimolar amounts of DNA of the various constructs (6.6 μ g pEJ (6.6); 13 μ g pMP-34.1; 16 μ g pNb-9; 1 μ g pMP-1) for two dishes which at the time of transfection contained 4×10^6 cells. About 24 h after transfection, the cells were split at a ratio of 1:3, re-fed at 3-day intervals with DMEM plus 5% and inspected for focus formation.

* For clones pMP-34.1 and pNb-9 see Fig. 1a. The mutated LTR/N-myc was generated by linearizing clone pMP-34.1^K at the single *Xho*I restriction endonuclease site (Fig. 1a), recutting the protruding single-stranded ends, followed by religation, thus creating a 4-base pair deletion. For clone pEJ (6.6) see ref. 11. Clone pMP-1 contains the U3 region of M-MuLV long terminal repeat (LTR).

† Based on three different experiments each involving $\sim 4 \times 10^6$ cells at the time of transfection.

clones pNb-9^K and pNb-9^N or cosmid clone pNb-101 (map not shown) had been introduced. We observed no obvious morphological differences between untransfected Rat-1 cells and those expressing high levels of human N-myc mRNA.

Previous studies¹⁰ had shown that second-passage rat embryo cells undergo neoplastic transformation when either a viral *myc* gene or a truncated *c-myc* from a murine plasmacytoma cell line, attached to a transcriptional enhancer, is co-transfected with c-Ha-ras-1(EJ). Co-transfection of rat embryo cells with the N-myc expression vectors pMP-34.1^K and pMP-34.1^N (Table 1) and c-Ha-ras(clone pEJ6.6; ref. 11) resulted in formation of foci between 7 and 10 days after transfection at a frequency of ~ 30 -40 foci per 4×10^6 cells. There was no obvious difference in the efficiency of N-myc genes derived from neuroblastoma line Kelly and of those from the normal individual. We did not obtain focus formation with any one of the control clones (Table 1). In particular, focus formation did not occur when we used an N-myc expression vector containing a frameshift mutation within N-myc, showing that enhanced expression of the product encoded by N-myc is required for neoplastic transformation.

Closer inspection revealed two morphologically different types of foci (Fig. 2): type I consisted of disorganized, loosely arranged, small, round to ovoid, refractile cells (Fig. 2a). The cells never reached high density within the focus, but floated off the bottom of the plate. Cells of individual foci grew in a three-dimensional pattern to high density (up to 2×10^8 cells per 100-mm dish (Fig. 2d,f)). Foci of type II consisted of elongated fibroblastic cells which grew in an organized pattern tightly attached to the surface of the plate (Fig. 2c). Individual foci grew into a flat monolayer (density up to 2×10^7 cells per 100-mm dish; Fig. 2e, g). The fraction of type II foci amounted to ~ 10 -20% of the total number of foci. The emergence of two different types of foci following co-transfection of N-myc and c-Ha-ras-1(EJ) cannot be fully explained at present. These different morphologies have not been reported for experiments in which *c-myc* or *v-myc* were used¹⁰. It is possible that different levels of N-myc and/or Ha-ras mRNA account for the different phenotypes of neoplastically transformed cells. Another possibility is that cells of different embryonic lineages present in the second-passage embryo cell cultures assume different phenotypes following uptake of N-myc and c-Ha-ras-1(EJ). Cells of all foci could readily be established as permanent lines, and all cells of individual foci appeared to start growing without delay.

Approximately 10^4 cells of foci of type I, when injected subcutaneously or intraperitoneally into athymic mice and

isogenic rats, formed tumours that caused death within 2-3 weeks. The results were not affected by irradiation of the animals before injection of the cells. Cells of type II foci did not yield tumours even when 5×10^7 cells were injected into X-ray-irradiated animals.

Cells of eight foci selected from two independent transfection experiments all contained 15-30 copies of N-myc and 10-20 copies of c-Ha-ras-1 (data not shown). Cells of all foci contained a major N-myc mRNA of 4.0 kb. The concentration of N-myc mRNA varied considerably among cells derived from different foci but did not correlate with the amount of N-myc DNA. Cells of type I foci generally contained high concentrations of N-myc mRNA (Fig. 3, lines VI-a, -d, -e and -k; VII-b, -c), as much as

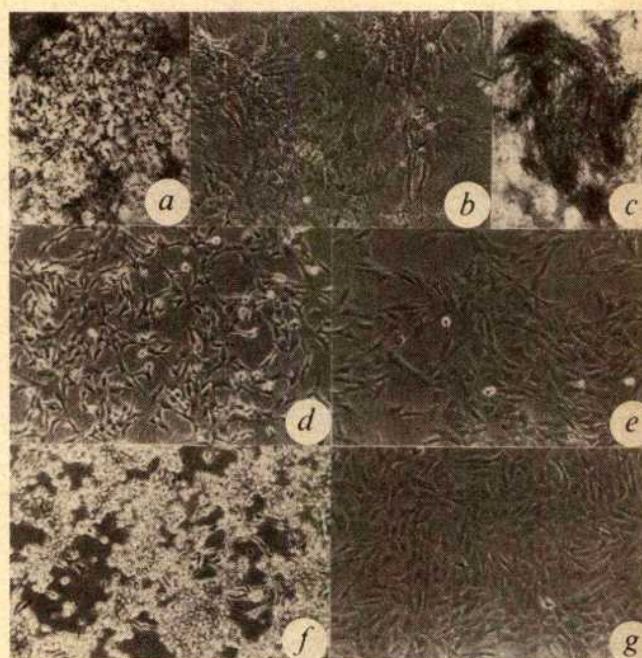


Fig. 2 Photomicrographs of rat embryo cells showing normal cells (b), and the morphology of foci of type I (a) and type II (c), resulting from transformation by LTR/N-myc and c-Ha-ras-1 (EJ). d, f, Transformed cells of focus type I (cell line VI-d) at low and high density, respectively. e, g, Cells of focus type II (line VI-j) at low and high density, respectively.

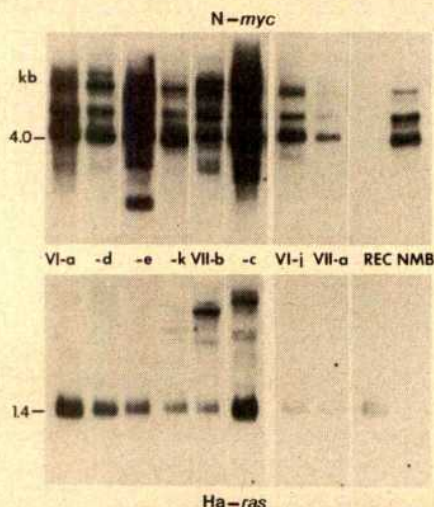


Fig. 3 Expression of *N-myc* and *c-Ha-ras* in rat embryo cells transformed by LTR/*N-myc* and *c-Ha-ras*-1 (EJ). Individual foci of transformed cells were cloned and grown into mass culture. Total polyadenylated RNA was isolated and analysed by agarose gel electrophoresis. VI-a, -d, -e and -k, and VII-c are cells derived from type I foci; VI-j and VII-a are cells from type II foci. REC, rat embryo cells; NMB, human neuroblastoma cell line. Each lane contains ~5 µg of RNA. The probe for *N-myc* was the *Eco*RI/*Bam*HI insert of pNb-1; for *ras*, the *Eco*RI insert of clone BS9 was used¹⁹.

10 times the level found in the neuroblastoma line NMB. Cells of type II foci generally contained lower levels of *N-myc* mRNA (lines VI-j, VII-a) that resembled the levels in neuroblastoma cell line NMB, although the level was in some instances only slightly lower than in type I cells (compare VI-d or -k with VI-j). There was apparently no absolute correlation, however, between the transformed phenotype of the cell and the level of *N-myc* mRNA: the level in the highly tumorigenic line VI-d was only slightly higher than that in the non-tumorigenic line VI-j.

In contrast, there seemed to be a correlation between the level of *Ha-ras* mRNA and the cellular morphology (Fig. 3). Levels of mRNA in cells of type I foci were three to five times higher than in rat embryo cells; in cells of type II foci, the level was not detectably higher than in rat embryo cells. The implications of this correlation are not clear. One possibility being tested is whether *N-myc* is capable of immortalizing rat embryo cells in culture.

N-myc, *c-myc* and *v-myc* apparently contribute in similar ways to transformation of early-passage embryo cells. The fact that at least one neuroblastoma cell line carries amplified *c-myc* instead of amplified *N-myc*¹² and, conversely, that several lines of cells from small-cell carcinoma of the lung have amplified *N-myc* instead of amplified *c-myc*⁷, indicates that there may also be a functional relation between *N-myc* and *c-myc* in the aetiology of naturally occurring tumours. It is not clear, however, that the similarities among the nucleotide sequences of *N-myc*, *c-myc* and *v-myc* are responsible for the apparent functional relationships. The function of *myc* genes in neoplastic transformation of cultured cells apparently can also be assumed by genes whose protein products are related to that of *myc* in regions other than the *N-myc/c-myc* similarity¹³, such as adenovirus early region 1A (ref. 14), or by genes whose relation to *myc* is not clear at present, such as those encoding the polyoma large-T antigen¹⁴ or the cellular tumour antigen, p53¹⁵⁻¹⁷.

We have found previously that enhanced expression and amplification of *N-myc* are correlated with stage III and IV neuroblastomas, characterized by poor prognosis⁸. It is not clear how the biological activity of *N-myc* in cultured cells relates to amplification of the gene in advanced neuroblastomas. The assessment of the role of *N-myc* in human neuroblastoma is complicated by the fact that classification of a specific stage of neuroblastoma is mainly based on the clinical picture at the

time of diagnosis rather than on cellular parameters; moreover, it is not known whether tumours of higher stages develop from lower-stage tumours. The correlation of *N-myc* amplification with stage III and IV neuroblastomas may indicate, therefore, that neuroblastomas comprise several classes of tumours, only one of which is characterized by *N-myc* amplification. We suggest this because primitive neuroectodermal tumours are characteristically difficult to distinguish from one another¹⁸; the heterogeneity among neuroblastomas now suggested by amplification of *N-myc* in only some tumours could therefore have gone undetected by more conventional criteria.

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Note added in proof: In a separate set of experiments we have now shown that *N-myc*, when co-transfected into rat embryo cells together with a gene conferring resistance against neomycin, is capable of both inducing an increased number of neomycin-resistant cells and establishing, for more than 120 cell divisions so far, host cells with morphology similar to that of cells derived from type II foci. These cells have a higher serum requirement than those derived from type II foci (details will be published elsewhere).

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The class I major histocompatibility antigen gene activated in a line of SV40-transformed mouse cells is *H-2D^d*, not *Qa/Tla*

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We have previously described several complementary DNA clones isolated because they correspond to messenger RNAs present at higher levels in the simian virus 40 (SV40)-transformed BALB/c 3T3 cell line SV3T3 Cl38 than in the normal, parental BALB/c 3T3 line¹. One of these clones, pAG64, hybridizes to RNAs which, while present in BALB/c 3T3 cells, are 10-20-fold more abundant in SV3T3 Cl38 and are found at high levels in a wide variety of transformed cell lines^{1,2}. Nucleotide sequence analysis showed that pAG64 encodes a class I antigen of the major histocompatibility

complex². To ascertain the identity of pAG64, we compared its sequence with the available sequences of *d* haplotype class I antigen genes [*K* locus³, *L* locus⁴, *D* locus⁵ and the *Qa* gene defined by genomic clone 27.1 (ref. 6)] and found that it showed multiple clustered differences from each of these sequences². We therefore concluded that it was not derived from the *H-2K^d*, *H-2L^d* or *H-2D^d* genes and thus must correspond to one of the other class I antigen genes, namely those of the *Qa/Tla* complex², although it was clearly not the *Qa* gene defined by the genomic clone 27.1. We now report subsequent findings which indicate that pAG64 in fact corresponds to the *H-2D^d* gene and not to a *Qa/Tla* gene.

We synthesized an oligonucleotide (nucleotides 716-733 in the sequence of pAG64) which should distinguish pAG64 from the other sequenced class I antigen genes of the *d* haplotype and used it to screen the BALB/c cosmid library of Steinmetz *et al.*⁷. We found that the oligonucleotide hybridized to the cosmid c18.1, which carries the *H-2D^d* gene, and to the cosmid 65.1 which carries two genes, 3 and 4 of cluster 1, from the *Qa2,3* region. Hybridization to the *H-2D^d* gene was unexpected in view of the sequence of this gene published by Margulies *et al.*⁵. Sher *et al.* have now reported⁸ the sequence of the cosmid c49.2, which carries the coding sequence of the *H-2D^d* gene. It is essentially identical to that of pAG64. Our conclusion that pAG64 did not correspond to *H-2D^d* was based on the sequence of the genomic clone Ch4a-D^d (ref. 5). Margulies *et al.* now report that the published sequence of this clone is incorrect⁹. They have re-determined the sequence of part of this clone and find that of 821 nucleotides sequenced, 821 are identical to the sequence of Sher *et al.* and that in the region of overlap, 299 nucleotides out of 300 are the same as pAG64. We therefore conclude that pAG64 corresponds to the *H-2D^d* gene and not to a *Qa/Tla* gene.

The other reason that led us to assign pAG64 to *Qa/Tla* was our inability to detect homologous transcripts in various normal mouse tissues which express *H-2* but not *Qa* and *Tla* antigens. We now know that this was an artefact of our mRNA isolation procedure. All of the samples used in our previous publications were prepared by lysing the cells in a buffer containing Nonidet P-40, separating nuclei from cytoplasm and extracting RNA from the cytoplasmic fraction¹⁰. However, when we extract total RNA by the guanidine isothiocyanate procedure¹¹, we observe, as expected, appreciable levels of RNA homologous to pAG64 in various mouse tissues. We had always reprobed our blots with a cDNA clone for the mitochondrial transcript encoding cytochrome oxidase subunit 1 and had observed a clear band of the expected size and intensity. Therefore, it seems that the use of mitochondrial probes does not represent an adequate control. We stress that, irrespective of the method of RNA isolation, we observe much higher levels of class I mRNA homologous to pAG64 in SV40-transformed cells than in their normal parents. Similar results have since been reported by others for other virus-transformed and virus-infected cells^{12,13}.

We conclude that pAG64 is a cDNA clone corresponding to the *H-2D^d* gene and that high levels of this RNA are present in the SV40-transformed cell line SV3T3 Cl38. Whether the abundant class I antigen transcripts detected in other transformed cell lines are all derived from the *H-2D* gene is not known. Although our discussion of the possible roles of *Qa/Tla* antigens in normal and transformed cells² should be disregarded, the induction of class I antigen gene expression in transformed cells is clearly of interest and we are presently analysing the mechanisms involved¹⁴.

We regret the confusion that the mis-assignment of this clone has caused.

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An enhancer-like sequence within the *Xenopus* U2 gene promoter facilitates the formation of stable transcription complexes

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Enhancers are eukaryotic promoter elements that increase transcriptional efficiency in a manner relatively independent of their position and orientation with respect to a nearby gene¹⁻³. There is growing evidence that enhancer action is mediated by *trans*-acting factors⁴⁻⁸, but the mode of action of these factors is not yet known. We report here on the *Xenopus* U2 gene promoter, which contains two sequence elements. The distal sequence element increases promoter activity 20-fold by facilitating the formation of stable transcription complexes. A synthetic 14-base-pair (bp) oligonucleotide corresponding to part of the distal sequence element, which shows homology to an immunoglobulin gene promoter element and to both the simian virus 40 (SV40) and the immunoglobulin heavy-chain gene enhancers, stimulates transcription in an orientation-independent manner.

The promoter of the *Xenopus* U2 snRNA gene¹⁰, in common with other vertebrate U snRNA genes¹¹⁻¹³, has no sequences homologous to the standard elements (TATA box, CCAAT box) found in the 5' control regions of most RNA polymerase II transcribed genes (reviewed in ref. 14). Pioneering studies on the human U1 gene promoter^{12,15} have shown that sequences essential for transcription of this gene in *Xenopus* oocytes lie between 203 and 230 bp upstream of the cap site. Recent work has shown that efficient transcription of a human U2 gene requires sequences located between 258 and 198 base pairs upstream of the cap site¹⁶, although 62 base pairs of 5' flanking sequence are sufficient to accurately direct transcription initiation in *Xenopus* oocytes⁹.

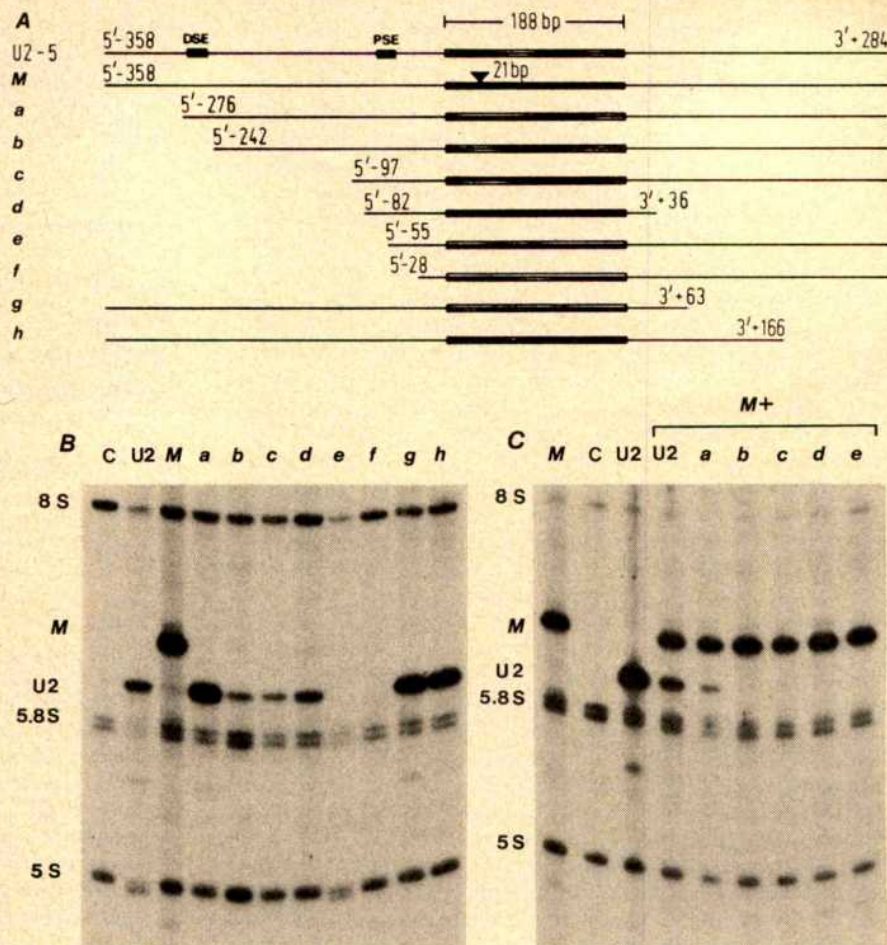
We have analysed the *Xenopus* U2 gene promoter by constructing a series of deletions removing parts of the 5' and 3' flanking sequences and assaying the transcription of the deletion mutants following microinjection into the nuclei of *Xenopus* oocytes. The extent of the deletions is shown in Fig. 1A. The gene indicated as *M* is a maxigene created by inserting 21 bp into the *Sau3A*I site between positions 28 and 32 of the U2 coding sequence¹⁰. The transcripts arising from mutants *a-h* are shown in Fig. 1B. Three conclusions can be drawn from the results shown in Fig. 1B. First, deletion of sequences between -82 and -55 (including the proximal element) eliminates U2 transcription (Fig. 1B, compare lanes *d*, *e*). Second, removal of sequences between -276 and -242 reduces the efficiency of transcription 10-20 fold (lanes *a*, *b*). No further reduction in transcriptional efficiency is seen on deletion of the sequences between -242 and -82 (lanes *b*, *c*, *d*). Third, deletion of sequences 3' to the U2 coding sequence up to 36 bp from the end of the coding sequence has no apparent effect on transcription (lanes *g*, *h*, *d*).

We next co-injected equal quantities of deletion mutant and

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Fig. 1 Transcriptional activity of mutant U2 genes with progressive deletions of flanking sequences. **A**, Diagrammatic representation of the extent of the various deletions. U2-5 was recloned from pXIU2-5 (ref. 10) into M13mp8 (ref. 28) and in this paper is referred to as wild-type U2. The 188-bp coding sequence is indicated as well as the length of flanking 5' and 3' sequence remaining. DSE and PSE are the distal and proximal sequence elements, and are described in the text. **M** is a maxigene made by the insertion of 21 bp into the U2 coding sequence. All the deletions are cloned in the same orientation in M13mp8 (ref. 28). **B**, Transcription of the U2 mutants after microinjection into *Xenopus* oocytes. Lane C, control oocyte, injected only with [α^{32} P]GTP. Lanes U2-h, oocytes injected with either U2 or one of the mutant U2 genes in Fig. 1A. The lanes are lettered corresponding to the mutant injected. U2 and maxigene transcripts are indicated, as are the endogenous 8S¹⁰, 5.8S, and 5S rRNAs. **C**, Transcription of U2 mutants after coinjection with the U2 maxigene. Lane C, control oocyte. Lanes U2 and M, oocytes injected with only U2 or maxigene DNA. Lanes M+U2 to M+e, oocytes coinjected with an equal amount of maxigene DNA and either U2 or mutant a to e DNA.

Methods. The construction of mutants a-c and e-h is described elsewhere²⁹. Mutant d is a *Fnu*4HI restriction fragment of pXIU2-5¹⁰, repaired with T4 DNA polymerase and cloned into *Sma*I digested M13mp8 (ref. 28). The maxigene was constructed by sub-cloning the two *Sau*3AI fragments of pXIU2-5 separately into the *Bam*HI site of M13mp8, excising them with either *Eco*RI-*Hinc*II or *Hind*III-*Sma*I double digests, and ligating them back into *Eco*RI-*Hind*III digested M13mp8 in their original configuration with the insertion of an *Xho*I linker between the *Sma*I and *Hinc*II ends. Microinjection and transcript analysis were performed exactly as in ref. 10. Relative transcription levels were estimated by scintillation counting of excised bands.



maxigene DNA to have an internal standard when comparing the transcriptional efficiency of various mutants. The results of this experiment are shown in Fig. 1C. The wild-type U2 and the maxigene are roughly equally transcribed on coinjection (Fig. 1C, lane M+U2). Mutant a, deleted from -358 to -276, which shows wild-type activity when injected alone (Fig. 1B, lane a), can still compete, although with a reduced efficiency compared to wild-type U2, when coinjected with maxigene (lane M+a). Mutants b, c and d, all of which are transcribed although at a reduced level when injected singly (Fig. 1B, lanes b-d) do not produce transcripts detectable above the level of endogenous U2 when co-injected with maxigene (Fig. 1C, compare lane M with lanes M+b, M+c, M+d).

The *Xenopus* U2 genes are tandemly repeated¹⁰. A construct which includes all the sequences up to -652, that is, to the prior coding sequence, behaves identically to the U2 clone with 358 base pairs of 5' flanking sequence in both single injection and coinjection experiments (data not shown), indicating that all the 5' flanking promoter elements necessary for full transcriptional activity in oocytes lie between -358 and -1. These results suggest that there must be a factor in oocyte nuclei whose concentration is rate-limiting for U2 gene transcription in these experiments. Sequences upstream of -242 are necessary for efficient association with this factor, such that when a gene with these sequences and a mutant in which they have been deleted are placed in direct competition, only the gene with the distal sequence element will be transcribed. In the absence of competition the factor can weakly associate with mutant genes lacking the distal sequence element, resulting in a lower level of transcription.

Mutant genes having only the proximal sequence element are thus weakly transcribed. We have made promoter constructs in which the proximal sequence has been deleted but in which the distal sequence element remains. These do not give rise to transcripts (data not shown) showing that the proximal sequence element is essential for transcription.

A characteristic of transcription complexes formed on genes transcribed by RNA polymerases III^{17,18} and I¹⁹ is that once formed, they are stable. Transcription factors remain closely associated with their DNA binding sites during many rounds of transcription. The competition experiments described above suggest that the distal sequence element is necessary for the association of a transcription factor. To test whether this association is also stable (through many rounds of transcription), competition experiments were performed in which two U2 constructs were microinjected consecutively rather than simultaneously. The first four lanes of Fig. 2 show that prior injection of vector DNA has no effect on the transcription of subsequently injected U2, maxigene, or mutant d DNA. This means that factors required for transcription do not associate stably with vector DNA. When U2 and maxigene DNA are coinjected, both are equally transcribed (Fig. 1C, lane M+U2). When they are consecutively injected, however, only the DNA injected first is efficiently transcribed (Fig. 2, lanes M+U2 and U2+M). This result suggests that transcription factors, at least one of which is limiting in concentration in these experiments, associate with the first injected DNA during the 3-h incubation, and that these complexes do not dissociate significantly during the following 20 h, that is, during many rounds of transcription. We estimate from Northern hybridizations that at least 10 transcripts per

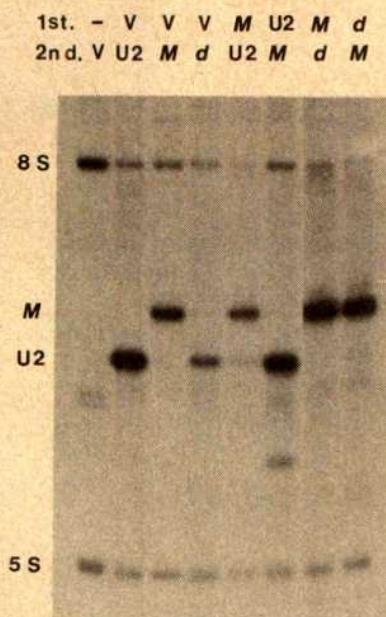


Fig. 2 Sequential injection of wild-type and mutant U2 genes. Oocytes were microinjected into the nucleus with one DNA, incubated for 3 h, microinjected with a second DNA, incubated for a further 3 h, then microinjected into the cytoplasm with [α^{32}]GTP. Twenty hours later RNA was extracted and analysed from single oocytes. The DNAs injected first and second are indicated. V, vector (M13mp8) DNA; U2, U2 DNA; M, maxigene DNA; d, mutant d DNA. Although only a single oocyte for each combination is shown, between 10 and 30 single oocytes were analysed in each case. The oocytes shown are representative of the most frequent (at least 70%) result obtained with each combination of injections. Some oocytes do not conform to the most frequent result, due to inaccurate microinjection such that some or all of one of the DNAs injected was not deposited in the nucleus, and thus was not transcribed. The main result, that genes lacking the distal sequence element cannot form stable transcription complexes, was obtained in three different experiments involving analysis of 30 individual oocytes.

injected gene accumulate during a 20-h incubation (data not shown). As the proportion of the injected genes assembled into active transcription complexes is unknown, the actual figure of transcripts per active gene could be significantly higher.

To determine the sequence requirements for this stable binding, we injected consecutively maxigene and mutant DNAs. The results for mutant d (Fig. 2) and mutant b (not shown), both of which lack the distal sequence element, were identical. Independent of whether the mutant DNA was injected before or after the maxigene, only the maxigene was efficiently transcribed (Fig. 2, lanes M + d and d + M). This shows that sequences >242 bases upstream of the cap site, likely to be identical to the distal sequence element identified in the previous section, are required for the formation of stable transcription complexes.

A sequence comparison of the region between positions -242 and -358 with other sequenced U snRNA genes revealed that homologous sequences are found in a *Xenopus* U1 gene³⁰ and a human U1 gene^{12,15} (Table 1). The human homology is part of the stretch between -203 and -230 previously shown to be necessary for transcription of this gene in *Xenopus* oocytes¹⁵. The *Xenopus* U1.3 gene can compete efficiently with the U2 gene on coinjection. Removal of sequences upstream of -216 from XIU1.3, including the homologous region shown in Table 1, results in the loss of this competitive ability (data not shown). A related sequence occurs in the promoter of immunoglobulin heavy-chain genes^{20,21} (Table 1), and in the promoters of immunoglobulin light-chain genes in the opposite orientation^{20,21}. A strongly homologous sequence is also present in the SV40 enhancer^{21,22} and, in the opposite orientation, in the

Table 1 A conserved region of the distal sequence element

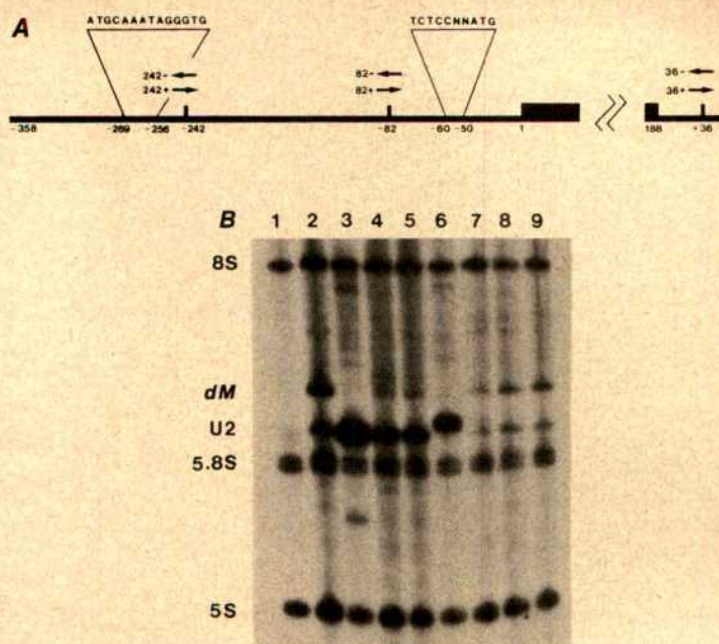
XIU2-5	AAGAGGCGGGGCTATGCAAAT-AGGGTG	-256
XIU1.5	TTGAAAAT-AGTCTG	-261
H.U1	ATGTAGATGAGGCAG	-205
Mammal Ig heavy-chain promoter	ATGCAAATNA	-60
SV40 enhancer	ATGCAAAGCA	
Mammal Ig heavy-chain enhancer (inverted)	ATGCAAATTA	
Synthetic oligonucleotide	GATCCATGCAAAT-AGGGTG GTACGTTTA-TCCACCATG	

Comparison of part of the U2 gene promoter with sequences found in a human U1 gene¹² and a *Xenopus laevis* U1 gene³⁰. They are compared with sequences found in the 5' flanking region of immunoglobulin genes of mice and men^{20,21} and of the SV40^{21,22} and immunoglobulin enhancers. The numbers on the right indicate the distance from the transcription initiation site. The sequence of a synthetic oligonucleotide, designed to test the functional importance of the conserved sequence is also shown. The *Xenopus* U2 sequence is extended to the left to indicate the extent of sequence conservation between *Xenopus*¹⁰, rat²⁷ and human^{9,16} U2 gene promoter sequences. This extended homology is not found in the U1 genes so far sequenced.

immunoglobulin heavy-chain gene enhancer⁸.

To test directly whether this homology is functionally significant, two 19-b complementary oligonucleotides, corresponding to the 14 b between -269 and -256 of the U2 promoter flanked by *Bam*HI linker ends (Table 1), were synthesized. This oligonucleotide duplex was then inserted into two mutants, b and d, both of which lack the distal sequence element. The site of insertion in mutant b, -242 (Fig. 3A), was such that the oligonucleotide was restored close to its original position. The sites of insertion into mutant d were at -82 in the 5' flanking region and +36 in the 3' flanking region (Fig. 3A). The orientation of insertion was determined by DNA sequencing and the clones designated 242+, 242-, 82+, 82-, 3'/36+ and 3'/36-, a plus sign indicating that the insert had the same orientation as in wild-type U2, a minus sign that the insert was inverted (represented by arrows in Fig. 3A). We then coinjected these DNAs with an equal concentration of either maxigene M (Fig. 1A), which has a complete promoter, or a new maxigene construct called dM which was made by joining the promoter of mutant d (Fig. 1A) to the coding region of the maxigene. Mutant dM therefore lacks the distal sequence element. Figure 3B shows the results of competition experiments with this gene. On coinjection, mutants d and dM are equally transcribed (Fig. 3B, lane 2), as are mutants b and dM (not shown). This is expected as these clones all lack the distal sequence element but retain the proximal sequence element. On coinjection of U2 and dM, only U2 transcripts are seen (Fig. 3B, lane 3), because U2 contains the distal sequence element. Mutants 242+ and 242- both show intermediate behaviour, being expressed to a much greater extent than dM regardless of the orientation of insertion of the synthetic oligonucleotide, but a small amount of dM transcription is still detectable (Fig. 3B, lanes 4, 5). These results indicate that insertion of the 14-b synthetic oligonucleotide, corresponding to part of the U2 promoter (Fig. 3A), into a site close to its natural position, results in partial complementation of distal sequence element function. This complementation is independent of the orientation of the insertion of the oligonucleotide despite the fact that it shows no rotational symmetry. To test whether the oligonucleotide would function independently of the position of insertion we next coinjected dM with clones 82+, 82-, 3'/36+ and 3'/36-. The result in Fig. 3B, lane 6 shows that clone 82+ competes efficiently with dM (more efficiently than either 242+ or 242-). The transcript produced

Fig. 3 The *Xenopus* U2 gene. The 188-bp coding region is indicated by the interrupted black rectangle. The positions of conserved sequences representing at least parts of the distal and proximal promoter sequence elements are shown. The TCTCCNNATG sequence was described²⁹ as a sequence element conserved in all *Xenopus* U snRNA genes so far analysed. The arrows above -242, -82 and +36 represent the position and orientation of insertion into the flanking sequences of a synthetic oligonucleotide described in the text and Table 1. **B**, Insertion of a 19-bp synthetic oligonucleotide restores distal sequence element function in an orientation independent manner. Mutant *dM*, constructed by combining the promoter of mutant *d* with the coding sequence of the maxigene through the *Nco*I site at -55, was coinjected with either U2, mutant *d* or a series of clones constructed by inserting a 19 bp synthetic oligonucleotide duplex into mutants *b* and *d* and termed 242+, 242-, 82+, 82-, 3'/36+ and 3'/36- depending on the position and orientation of insertion of the oligonucleotide (Fig. 3A). A plus sign indicates that the oligonucleotide is in the same orientation as in U2-5, a minus sign that it is in the inverse orientation. The two DNAs were coinjected at equal concentration. Lane 1, control oocyte; lane 2, *dM*+*d*, lane 3, *dM*+U2; lane 4, *dM*+242+; lane 5, *dM*+242-; lane 6, *dM*+82+; lane 7, *dM*+82-; lane 8, *dM*+3'/36+; lane 9, *dM*+3'/36-. The U2 and *dM* transcript sizes are indicated. Equal transcription of *dM* and the competitor clone indicates that neither clone can stably bind to a limiting transcription factor. Excess of transcription over *dM* indicates that the competitor clone can bind the factor required for the formation of stable transcription complexes.



from this mutant is slightly longer than U2, which probably results from the close proximity of the distal and proximal sequence elements in this construct affecting the accuracy of transcription initiation. Mutant 82- is transcribed equally to *dM* (Fig. 3B, lane 7) indicating that at this position insertion of the oligonucleotide in inverted orientation has no effect on promoter efficiency. Similarly, insertion of the oligonucleotide in either orientation into the 3' flanking region in clones 3'/36+ and 3'/36- has no effect on promoter efficiency (Fig. 3B, lanes 8, 9). These results were confirmed by testing the oligonucleotide-containing genes against maxigene *M* (Fig. 1A). 242+, 242- and 82+ could all compete to a limited but significant extent with maxigene *M*, whereas 82-, 3'/36+ and 3'/36- could not (data not shown).

Insertion of the synthetic oligonucleotide in either orientation near to its natural position (constructs 242+ and 242-) or 170 base pairs nearer to the cap site in the normal orientation (82+) therefore results in partial restoration of the competitive ability of mutants *b* and *d*. The reason for the restoration being incomplete is probably because the 14 bp of promoter sequence inserted do not represent the entire distal sequence element, but only part of it. In this context the behaviour of mutant *a* in competition with the maxigene is of interest. This mutant, deleted from -358 to -276, to within 7 bp of the homology shown in Fig. 3A, competes less efficiently than U2 with the maxigene (Fig. 1C compare lane *M*+*a* with lane *M*+U2). An explanation for this would be that part of the distal sequence element has been removed in this mutant.

The independence of orientation and, to some extent, of position of insertion of the oligonucleotide suggests that the distal sequence element is enhancer-like. It has been reported previously that viral enhancers are not completely position independent^{2,3}. As the oligonucleotide seems to represent only part of the promoter element this might mean that it would be more subject to effects of the sequences flanking its insertion site. The fact that 82+ competes more efficiently than either 242+ or 242- with both maxigene and mutant *dM* (compare lane 6 with lanes 4, 5 in Fig. 3B) suggests that such position effects can be positive as well as negative.

The *Xenopus* U2 gene promoter contains two elements necessary for maximal transcriptional activity in *Xenopus* oocytes. The distal of the two elements is required for full promoter efficiency, while the proximal sequence element is

essential for transcription. The bipartite nature of the U2 promoter is consistent with results obtained by other laboratories working with U snRNA gene promoters^{9,15,23,24}.

The formation of stable transcription complexes has been shown to be an important step in transcription by RNA polymerases I and III¹⁷⁻¹⁹. In the case of RNA polymerase III transcription the DNA sequences necessary for stable complex formation are internal to the genes¹⁷. The DNA element necessary for stable RNA polymerase I complexes is an enhancer, that is, is orientation and position independent²⁵. The results presented here show that the formation of stable transcription complexes is also a step in RNA polymerase II transcription *in vivo*. In addition the DNA sequence element required for their formation acts independently of orientation, that is, it has some of the properties of an enhancer. This suggests that the function of enhancers in general is to promote the formation of stable transcription complexes, a hypothesis that could be tested experimentally with better characterized enhancers in other gene systems, such as SV40 or polyoma. The U2 distal sequence element function is partly restored by insertion of a synthetic oligonucleotide containing only 14 base pairs of U2 promoter sequence. This sequence does not display rotational symmetry, and does not appear to be repeated within the U2 tandem repeat unit. Surprisingly, the first 8 b of these 14 are identical to a sequence element found roughly 60 base pairs 5' of the cap site of mammalian immunoglobulin heavy-chain genes^{20,21} (Table 1). This element is found in the same position, but in the opposite orientation, in the promoter of immunoglobulin light-chain genes^{20,21}. This sequence also has a counterpart in the SV40 enhancer^{21,22} (Fig. 3A), and is also present, in the opposite orientation, in the immunoglobulin heavy-chain enhancer⁸ (Fig. 3A). In fact the G residue located in this sequence is protected against dimethyl sulphide modification in cells of the B lineage, but not in other cell lines⁸. This suggests that this sequence, within the immunoglobulin heavy-chain enhancer, interacts with a factor in B cells, and this is likely to be important in bringing about the cell-type specific expression of the immunoglobulin genes. These data support the notion that this element is a general, orientation independent promoter element. The eight absolutely conserved bases may represent the core of the element, whose activity could be modified by non-conserved sequences in the immediate vicinity. It may be that the distinction between enhancers and other upstream promoter elements will

become more tenuous as we understand more about how they function. Note that the first orientation-independent promoter element was found when a restriction fragment upstream of the sea-urchin H2A histone gene was inverted²⁸.

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Note added in proof: Recent experiments carried out in collaboration with Angelo Tognoni and Walter Schaffner have shown that in CV1 (Monkey) cells the *Xenopus* U2 distal sequence element can functionally replace the SV40 enhancer to allow replication and T-antigen expression in SV40 mutants lacking the SV40 enhancer.

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Amplification of *inter-Alu* extrachromosomal DNA during cellular ageing: retraction and explanation

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We reported previously^{1,2} the age-dependent appearance of extrachromosomal circular DNA bands hybridizing to a human DNA fragment ('*inter-Alu*'), isolated from a genomic cluster of *AluI* repeats. Such bands appeared or increased at late passage in four out of six human fibroblast strains (six out of nine cell expansions); moreover, all DNAs (9/9) obtained from peripheral lymphocytes of aged donors, but none (0/8) from young donors, revealed a non-genomic *inter-Alu* band at ≈ 4.8 kilobases (kb)^{1,2}. Subsequent data extended these numbers to 16/24 aged donors compared with 0/18 young donors³. These results were interpreted as evidence of age-dependent DNA rearrangement in normal human cells^{1,2}. We now report that the 'extra' bands were of microbial origin, although clearly occurring in an age-dependent manner.

The hybridizations reported^{1,2,4} primarily used the entire *inter-Alu*-containing plasmid, pAH15A, as ³²P-DNA probe. Gel-purified insert DNA also hybridized to the extrachromosomal species, which was then believed to be an adequate control for plasmid contamination. Such hybridization, however, could still be due to residual traces of vector DNA, "invariably present in [insert] probes ... even after two cycles of electrophoretic purification" (ref. 5). Control hybridizations against many of the same

DNA preparations, with a pCR1 fragment or pBR322 plasmid containing other inserts, revealed no extra bands. However, when we obtained the parental vector for *inter-Alu*, pACYC184, we observed hybridization of this probe to the age-dependent bands in both fibroblast and lymphocyte DNA samples, indicating that such bands must be due to low levels of microbial plasmid DNA ($\approx 10^{-6}$) in our human DNA samples. We discussed the dangers of microbial contamination¹, but were misled by unwarranted confidence in the controls, the pervasive extent and polymorphic nature of the contamination, and its remarkable age dependence.

In attempting to account for our data, we have considered the possibility of deliberate contamination and regard it as extremely unlikely. Inadvertent operator contamination, however, is difficult to reconcile with the marked age bias observed, in particular for the multiple fibroblast DNA bands seen in many independent samples, prepared by six persons over a 2-yr period. Biological contamination is more plausible in the light of recent reports of microbial plasmid DNA in biopsy and necropsy tissue samples and in body fluids⁵⁻⁷. Our preliminary evidence indicates that plasmid-homologous DNA can be detected in filter-sterilized serum and trypsin, two common reagents for cell culture. Thus, the correlation between culture age and plasmid bands may reflect cumulative exposure of fibroblasts to plasmid DNA.

We apologize for any inconvenience to other investigators resulting from this error. Our experience, along with other reports^{5,6,8}, underscores the danger of systematic plasmid contamination of DNA samples, and the necessity for control hybridizations using the appropriate vector alone as probe.

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In vitro reactivation of anaphase spindle elongation using isolated diatom spindles

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A key step for analysing the mechanochemistry of mitosis would be the isolation of a functional spindle capable of anaphase chromosome movement *in vitro*. Although Mazia and Dan first isolated spindles in 1952¹, with one or two possible exceptions²⁻⁴, isolated spindles are non-functional (reviewed in ref. 5). An alternative approach has used permeabilized cells to study anaphase chromosome movement⁶⁻¹⁰, but these preparations are biochemically and morphologically complex, and hence difficult to analyse. We describe here a simple procedure for isolating diatom spindles which are capable of anaphase spindle elongation *in vitro*. With addition of ATP, the two half-spindles slide completely apart, with concomitant decrease in the zone of overlap. Electron microscopy reveals decreased numbers of microtubules throughout the spindle after ATP addition and confirms the complete absence of structures beyond the spindle poles. These results are inconsistent with theoretical models of mitosis which suggest that spindle poles are pushed apart by microtubule growth¹¹, are pulled apart by external forces applied to the poles¹²⁻¹⁵, or are

released from tension generated during spindle formation¹⁶. The results are consistent with models that postulate mechanical interactions in the zone of microtubule overlap as a factor in spindle elongation^{17,18}.

Diatom spindles are important model systems for studying the morphological changes associated with anaphase chromosome movement because the central spindle microtubules are arranged in paracrystalline arrays and the microtubules associated with chromosome-to-pole movement (anaphase A) are spatially separated from the microtubules associated with spindle elongation (anaphase B)^{19,20}. Morphometric analysis has shown that the diatom central spindle is constructed of two half-spindles which exhibit specific near-neighbor interactions in the zone of overlap²¹. This microtubule overlap zone, which is visible even by light microscopy, decreases as the central spindle elongates during anaphase B²².

The centric diatom *Stephanopyxis turris* was selected as starting material because it can be grown in large quantities in a simple medium and its cell cycle is easily synchronized (L. Wordeman, K.L.Mc.D and W.Z.C., in preparation). Following homogenization, spindles are isolated by a two-step procedure consisting of filtration to remove cell walls and low-speed centrifugation of spindles onto coverslips (Fig. 1). The central spindle is readily detected as a phase-dense bundle of fibres embedded in a tangled mass of chromatin (Fig. 1a). In polarization optics, the central spindle is a highly birefringent structure with a prominent zone of microtubule overlap, showing increased birefringence, which is 15-25% of the total spindle length (Fig. 1b, Table 1).

Addition of 1mM ATP by perfusion leads to an increase in

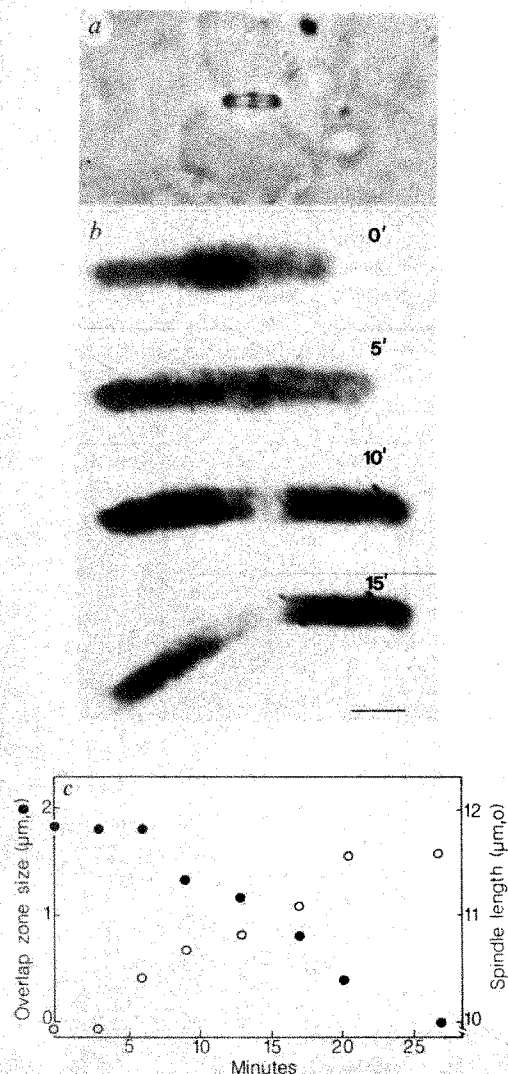


Fig. 1 Changes in spindle structure after reactivation as monitored by light microscopy. *a*, An isolated spindle viewed with phase optics. Note the prominent phase-dense overlap zone and the tangled mass of chromatin. *b*, An isolated spindle viewed with polarization optics with negative compensation, just before ATP addition (0 min) and after addition of 1 mM ATP (at 5 min, 10 min, 15 min). Note the decrease in the birefringence in the zone of microtubule overlap during spindle elongation. There is further loss of birefringence from the midzone even after elongation is complete. The spindle elongated at a rate of $0.3 \mu\text{m min}^{-1}$. Scale bar, $2 \mu\text{m}$. *c*, Change in extent of birefringence of the zone of microtubule overlap (●) during spindle elongation (spindle length; ○) on addition of 1 mM ATP at time 0. Midzone birefringence continues to decrease after elongation is complete. The rate of elongation in this example, which differs from *b*, is $\sim 0.1 \mu\text{m min}^{-1}$. Normal anaphase spindle elongation occurs at a rate of $1-2 \mu\text{m min}^{-1}$ and the increase in spindle length is much greater than the size of the overlap zone. Some of this length increase must be due to microtubule growth (data not shown).

Methods. Cells were grown in f/2 medium³⁰ in a suspension culture on 6-h-day, 18-h-night schedule at 19°C . Three hours before collection 2×10^{-8} M nocodazole was added and 20 min before collection, cells were placed on 60- μm mesh Nitex filters and washed extensively to remove the drug. Cells were collected by filtration at the height of the division peak which occurred $1\frac{1}{2}$ -2 h after night began. Cells were resuspended in homogenization medium at room temperature (50 mM PIPES, pH 7.0, 40 mM sodium glycerophosphate, 10 mM MgSO_4 , 10 mM EGTA, 0.2% Brij 58, 30% glycerol, 1 mM dithiothreitol (DTT) and inhibitors of proteolysis³¹, then homogenized on ice with two strokes of a Kontes Dounce homogenizer, B pestle. The homogenate was filtered once through 60- μm mesh and once through 25- μm mesh Nitex filters, then centrifuged for 20 min, 0°C at 2,000 g onto coverslips. Coverslips were mounted on slides, using strips of Parafilm as spacers, waxed down with Valap⁷, and then perfusion was performed as described previously for lysed cells⁸. Reactivation medium consisted of 50 mM PIPES pH 7.0, 5 mM MgSO_4 , 10 mM EGTA, 1 mM ATP, 1 mM DTT, with or without 7.5% glycerol. The progress of reactivation was monitored with Zeiss polarization optics, 40 $\times$ Nikon rectified lens, Bracke-Kohler A/30 compensator and a DAGE-MTI low light level television camera connected to a Panasonic time-lapse video recorder. Two KG1 heat filters, a 1.5-inch column of saturated copper sulphate solution, and a narrow-pass green interference filter were placed between the 100-W mercury light source and the condenser to minimize damage to the spindle. The isolation medium was designed to stabilize the microtubule components of the spindles, but it was necessary to include protease and phosphatase inhibitors to obtain reliable reactivation of spindle function. Although yield is low, the coverslips are covered with hundreds of spindles at a similar stage of mitosis (metaphase to early anaphase); the only contaminants present are interphase nuclei, chloroplasts and glass cell wall fragments.

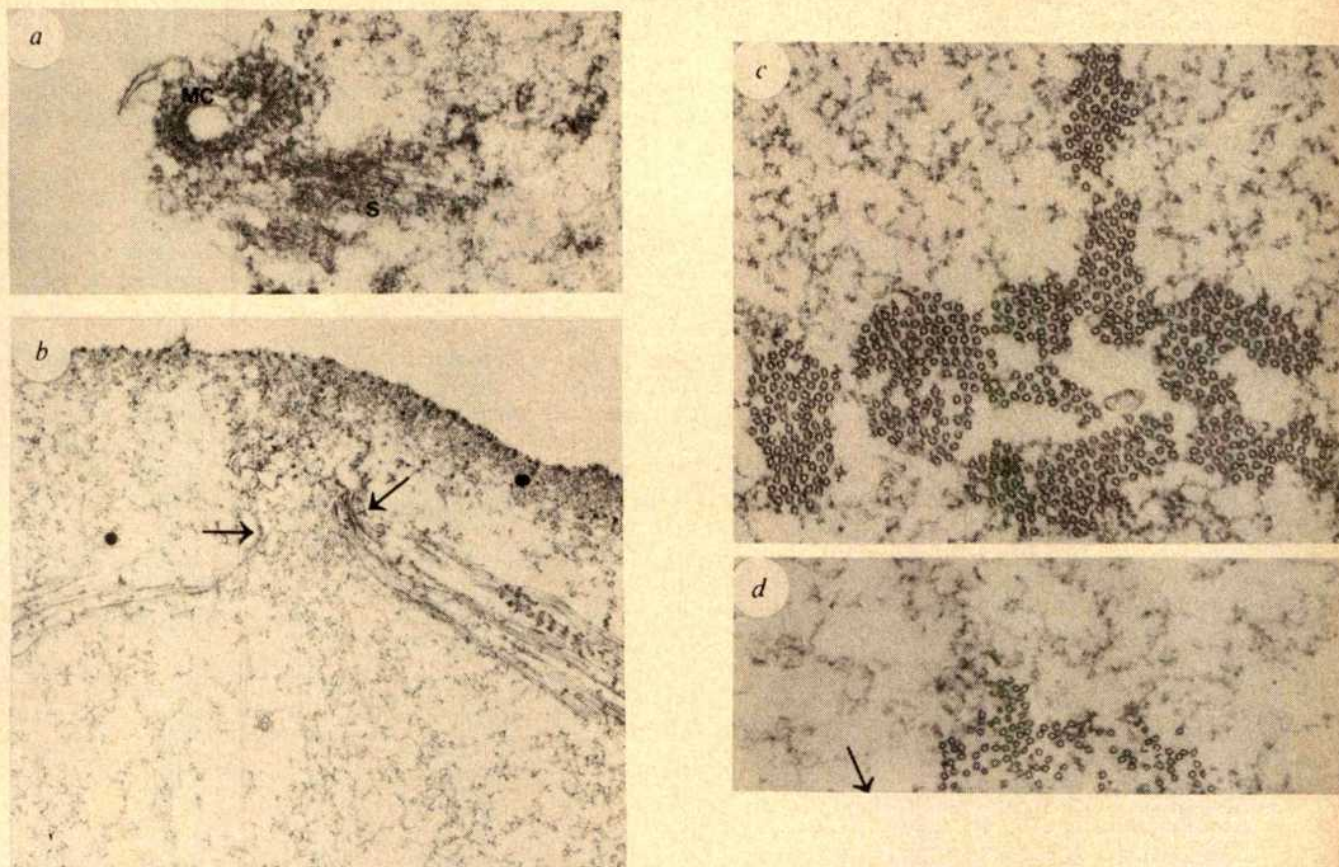


Fig. 2 Electron microscopy of isolated spindles with (*b, d*) and without (*a, c*) added ATP. *a*, Spindle pole structure showing the mitotic centre (MC) or centrosome at the end of the obliquely sectioned spindle (S). No cytoplasmic structures extend beyond the pole. $\times 28,500$. *b*, Longitudinal section through the middle of an isolated spindle after 10 min in 1 mM ATP. The few remaining MTs in this region are abnormally curved (arrows). $\times 11,000$. *c*, Cross-section through the overlap region in an isolated anaphase spindle (minus ATP). There are 623 hexagonally packed microtubule profiles in this section. $\times 41,000$. *d*, Cross-section through the overlap region in an ATP-treated spindle of comparable mitotic stage with the one in Fig. 2*c*. There are 108 microtubule profiles in this section. This spindle is very close to where the coverslip was (arrow) before it was dissolved with HF before sectioning. $\times 41,000$.

Methods. Isolated spindles attached to coverslips were fixed with 0.5% glutaraldehyde in f/2 medium³⁰ for 30 min at 23 °C (unless otherwise stated, all remaining steps were at 23 °C). Spindles were rinsed for 5 min in f/2, then 5 min in PBS (physiologically buffered saline: 137 mM NaCl, 2.68 mM KCl, 8 mM $\text{Na}_2\text{HPO}_4/\text{H}_2\text{O}$, 1.47 mM KH_2PO_4) at pH 7.3, fixed in 0.2% $\text{OsO}_4 + 0.3\%$ $\text{K}_3\text{Fe}(\text{CN})_6$ in PBS for 15 min at 4 °C, rinsed twice in PBS for 5 min each, rinsed in distilled H_2O for 5 min, incubated in 2% aqueous uranyl acetate for 90 min, dehydrated in acetone by 10% steps for 10 min each, and embedded in Epon-Araldite.

spindle length and a concurrent decrease in the magnitude and extent of birefringence in the zone of microtubule overlap (Fig. 1*b, c*). Within 5 min after ATP addition, spindles begin to elongate at a rate of 0.1–0.4 μm per min for several minutes. After spindle elongation is complete, the birefringence in the spindle midzone continues to decrease. In the example shown in Fig. 1*b*, the two half-spindles have become disengaged and the extent of birefringence of each half-spindle has shortened by $\sim 1 \mu\text{m}$.

For all of the spindles studied on-line by video microscopy (Table 1), we find that the increase in spindle length is about the same as the length of the microtubule overlap zone and that spindle midzone birefringence always continues to decrease after elongation is complete. The sample size in Table 1 is necessarily small because the isolated spindles are easily damaged by the manipulations required for on-line observations. It is easier to study the physiology of spindle elongation using populations of spindles (see Table 2). After incubation for 10 min in 1 mM ATP, >85% of the spindles on a coverslip show distinct midzone birefringence changes. Those spindles which display reduced birefringence are significantly longer than control (minus ATP) spindles or spindles which do not have reduced midzone birefringence.

Longitudinal sections and serial cross-sections of *in vitro* spindles show that the mitotic centre is retained during isolation (Fig. 2*a*) and that no other structures extend beyond the end of the isolated central spindle. The overlap zone is the region most drastically altered by ATP. Normally the most well-ordered part

of the spindle^{19–21}, the overlap is in complete disarray after 10 min in 1 mM ATP (Fig. 2*b*). Isolated spindles (minus ATP) were similar to *in vivo* spindles in the number of microtubules per half-spindle (~ 300) and extent of microtubule overlap at early anaphase ($\sim 20\%$). After 10 min in 1 mM ATP, the number of microtubules present drops to about 110–120 per half-spindle, and relatively few microtubules are found in the overlap region (Fig. 2*d*) compared with control spindles (Fig. 2*c*).

The newly formed gap between the half-spindles is not due just to microtubule depolymerization. The microtubules in the isolated central spindles are very stable and the birefringence pattern shown in Fig. 1*b*, 0 min, will persist for several hours in the absence of ATP. Addition of nonspecific agents which promote microtubule depolymerization, such as high salt, do not lead to gap formation, but rather to a decrease in overall birefringence and some spindle shrinkage. This shows that ATP addition leads not only to spindle elongation but also to a defined pattern of microtubule disassembly, starting from the midzone and working poleward. We suggest that the mechanochemical events leading to spindle elongation and the ATP-induced lability of microtubules *in vitro* are closely related. One possibility is that there is a discrete cap on the end of every microtubule in the overlap zone (the plus end^{23,24}) that regulates microtubule assembly–disassembly in an ATP-dependent manner. Alternatively, or in addition, lateral interactions between overlapping microtubules may stabilize them²⁰, and as the two half-spindles separate, giving less overlap, the microtubules begin to depolymerize. Either suggestion would be consistent

Table 1 *In vitro* spindle elongation monitored by video microscopy

Original spindle length (μm)	Final spindle length (μm)	Length change (μm)	Length of initial zone of microtubule overlap (μm)
9.3	11.0	1.7	1.8*
10.1	12.0	1.9	2.2*
6.3	7.5	1.2	1.1*
8.8	10.5	1.7	2.0†
8.3	10.0	1.7	2.0†
9.6	11.7	2.1	2.1†

* There was a distinct gap between half-spindles at the end of experiment.

† Spindle midzone was reduced in birefringence to same level as the rest of the half-spindle and the midzone was thinner or collapsed.

Table 2 Average spindle lengths of experimentally treated populations of spindles

Treatment	n	Average spindle length (μm)	Size of zone of microtubule overlap (μm)	Change in average spindle length (μm)
No ATP control	25	8.4 $\pm$ 0.9	1.9 $\pm$ 0.2	
Plus 1 mM ATP:				
Reduced overlap zone birefringence	26	10.7 $\pm$ 0.8*	†	2.3‡
No change in overlap zone birefringence	5	8.2 $\pm$ 0.6	1.7 $\pm$ 0.2	-0.2

Coverslips prepared as described in Fig. 1 legend were incubated for 10 min at room temperature in reactivation medium with or without ATP, then 0.5% glutaraldehyde was added. The lengths of individual spindles were measured off the TV monitor. All spindles on a transect of the preparation were used for these measurements, except it was necessary to disregard five or six spindles whose poles were not in good focus or were obscured by debris.

* The difference between the means (compared with no ATP) was statistically significantly different at $P < 0.001$ using Student's *t*-test.

† The average size of the gap between the two half-spindles was $0.7 \pm 0.3 \mu\text{m}$.

‡ As compared with average spindle length of control (minus ATP) spindles.

with the directed pattern of microtubule disassembly observed during telophase in the diatom *Pinnularia*²⁵ or after ultraviolet microbeaming of diatom spindles²⁶.

Anaphase chromosome movement is a complex process involving several distinct mechanochemical events (reviewed in refs 27, 28). Our observation of a loss of birefringence in the zone of microtubule overlap during reactivation of spindle elongation suggests that microtubule sliding is the key mechanochemical event that occurs during anaphase B. Ultraviolet microbeaming experiments on living dividing diatoms demonstrate that anaphase B *in vivo* also depends on the generation of sliding forces in the zone of microtubule overlap²⁹. Several investigators have suggested that dynein-like interactions between interdigitating sets of microtubules in the overlap zone may be responsible for spindle elongation^{17,18,20}. Although we cannot identify the motor, the requirement for ATP and inhibition of elongation by $10 \mu\text{M}$ vanadate (data not shown) suggests that a dynein-like ATPase may be involved. The observation that similar conditions affect spindle elongation in lysed mammalian cells¹⁰ suggests that an analogous mechanism may be important for anaphase B in a wide variety of cells.

The forces responsible for spindle elongation *in vitro* could not have been generated by microtubule polymerization¹¹, as no tubulin is present during reactivation. However, *in vivo* in this diatom and many other cells, microtubule growth is an integral part of anaphase B and leads to an increase in spindle length much greater than can be generated by microtubule rearrangements alone. The autonomy of the central spindle and

the lack of attachment of the spindle poles to any other cytoplasmic structures beyond the poles eliminates the possibility that the half-spindles are pulled apart by astral microtubule interactions with cytoplasmic components¹²⁻¹⁵. Although it has been suggested that spindle poles are self-propelled¹³, these phenomena may have more to do with nuclear migration and repositioning at the end of cell division than with anaphase B. Finally, it has been suggested that tension generated during spindle formation and released during chromosome-to-pole movement may be responsible for spindle elongation¹⁶. As little or no chromosome-to-pole movement occurs during spindle reactivation, this mechanism is also inconsistent with our observations.

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β -Hairpin families in globular proteins

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β -Hairpins, one of the simplest supersecondary structures, are widespread in globular proteins, and have often been suggested as possible sites for nucleation¹. Here we consider the conformation and sequences of the loop regions of β -hairpins by analysing proteins of known structure. We find that the 'tight' β -hairpins, classified by the length and conformations of their loop regions, form distinct families and that the loop regions of the family members have sequences which are characteristic of that family. The two-residue hairpin loops include almost entirely I' or II' β -turns, in contrast to the general preference for type I and type II turns^{2,3}. These findings are being used to help define templates or consensus sequences to be incorporated into our existing supersecondary structure prediction algorithm^{4,5}. This information can also be used in model-building homologous proteins.

Fig. 1 *a*, Distribution of the number of residues in the loop region for 107 β -hairpins. *b*, Schematic diagram of a β -hairpin. Data derived from the 62 proteins included in the Kabsch and Sander dictionary of secondary structure⁶. Loop residues are defined as those not participating in the antiparallel β -ladder⁶, which forms the hairpin. Residue 1 participates in the ladder if both its NH and CO groups form the standard antiparallel H-bonds, or if adjacent residues CO_{*i*-1} and NH_{*i*+1} form these bonds. If the first and last residue of the loop are linked by one hydrogen bond (NH_{*i*+1} to CO_{*i*-1}), this is denoted by an asterisk in Table 1. This definition is unequivocal and in practice is simple to apply to the Kabsch and Sander output. The ladder must include at least two residues in each strand, and only loops which do not include a helix or another strand are counted. For homologous loops (that is, >50% amino-acid identity), only one family member was included.

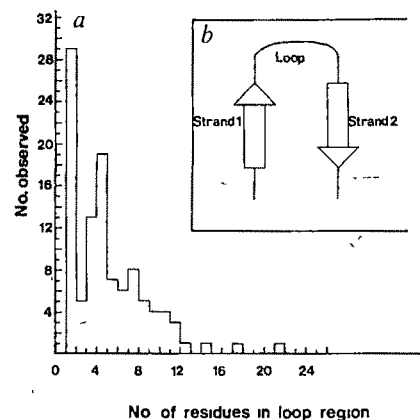
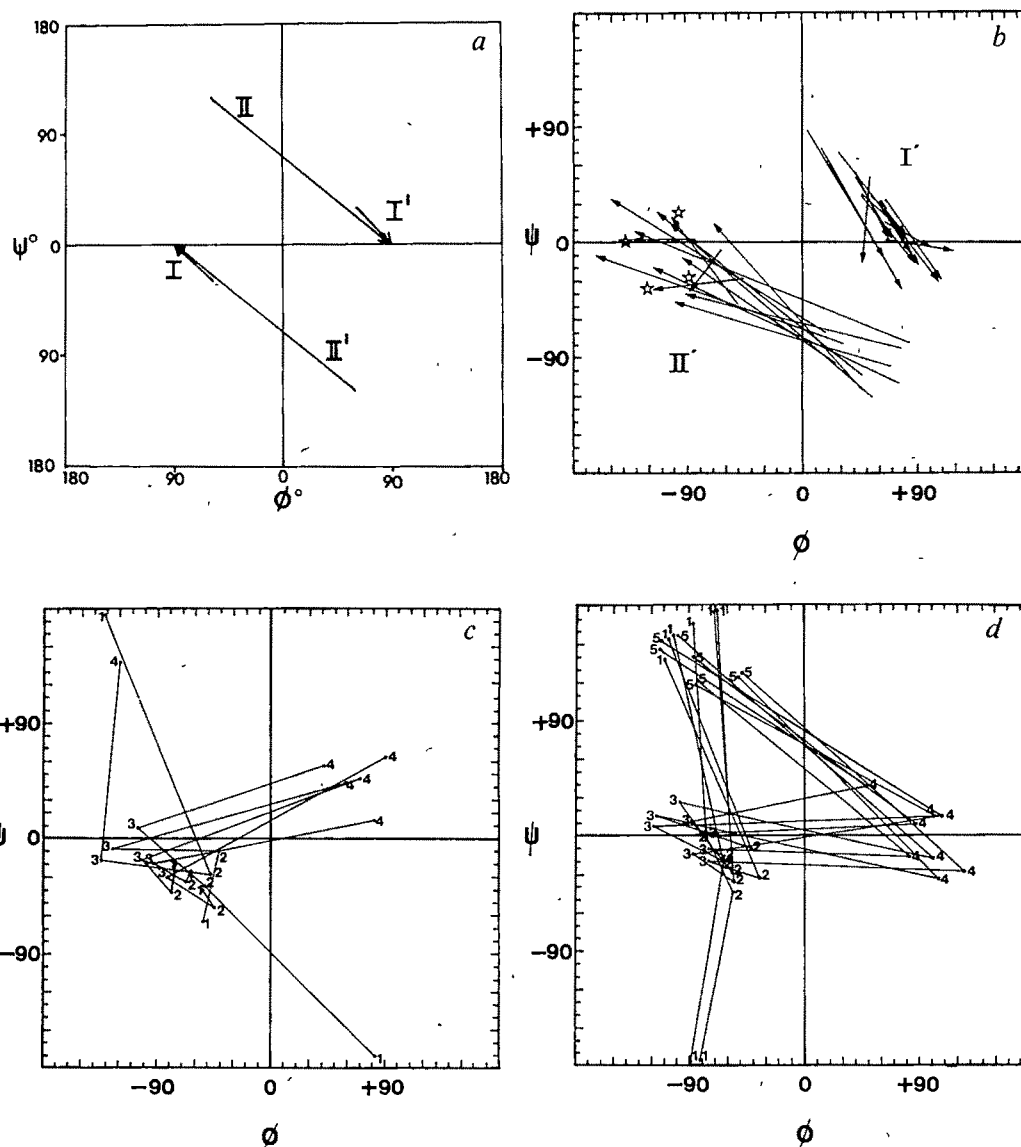


Fig. 2 Conformation of loop regions shown by plotting ϕ, ψ values for the loop residues. *a*, Classical β -turn conformation types I and II and their mirror images I' and II' taken from Chou and Fasman². These are defined by the ϕ, ψ values of the two central residues of the turn (usually referred to as residues 2 and 3) which in the context of, say, a two-residue hairpin, will be described as residues L1 and L2. The $\phi_{L1}, \psi_{L1}, \phi_{L2}, \psi_{L2}$ values plotted are type I = $-60^\circ, -30^\circ, -90^\circ, 0^\circ$; type I' = $+60^\circ, +30^\circ, +90^\circ, 0^\circ$; type II = $-60^\circ, 120^\circ, 90^\circ, 0^\circ$; type II' = $60^\circ, -120^\circ, -90^\circ, 0^\circ$. Since type III turns do not form a group distinct from the type I turns¹⁶, they have been omitted. Arrowheads denote the L2 ϕ, ψ value. *b*, ϕ, ψ Plot for the 29 two-residue hairpin loops given in Table 1. For each loop the ϕ, ψ values of residue L1 are connected by a line to the ϕ, ψ values of residue L2, with the arrowhead denoting the L2 position. Types I' and II' turns predominate, with only four type I turns, denoted by stars, and no type II turns, observed. *c*, ϕ, ψ Plot for the six four-residue hairpin loops with type I turns given in Table 1a. For each loop the ϕ, ψ values of residues L1, L2, L3 and L4 are plotted and joined together sequentially. Five of these hairpins also have residue L4 in the $+/+$ quadrant of the ϕ, ψ plot. The ϕ, ψ angles calculated by averaging over the four similar loops for residues L1 to L4 are $-62^\circ, -38^\circ, -58^\circ, -35^\circ, -101^\circ, -19^\circ, 75^\circ, 42^\circ$. *d*, ϕ, ψ Plot for the eight five-residue hairpin loops which form a family (see Table 1a). For each loop the ϕ, ψ values of residues L1, L2, L3, L4 and L5 are plotted and joined together sequentially. Note the type I turn for residues L2 and L3 and the positive ϕ values for residue L4. The standard ϕ, ψ angles for residues L1 to L5, calculated by averaging over the eight hairpins in the family, are $-90^\circ, 161^\circ, -56^\circ, -24^\circ, -92^\circ, 2^\circ, 95^\circ, 2^\circ, -85^\circ, 136^\circ$.



A β -hairpin involves two consecutive β -strands that lie antiparallel and are linked by hydrogen bonds (Fig. 1). The connecting loop region may or may not include a classical β -bend^{2,3}. To analyse the number of residues in the loop region, we used the non-subjective assignments of β -strands and coil regions given for the 62 proteins included in Kabsch and Sander's

dictionary of secondary structures⁶. Thirty-nine proteins had β -hairpins, giving a total of 107 hairpins. Loop residues (referred to as L1, L2, ...) are defined as those not participating in the antiparallel β -ladder which forms the hairpin (see Fig. 1 legend). The residues of the hairpin strands are denoted by ...-B3, -B2, -B1 for the N-terminal strand and +B1, +B2,

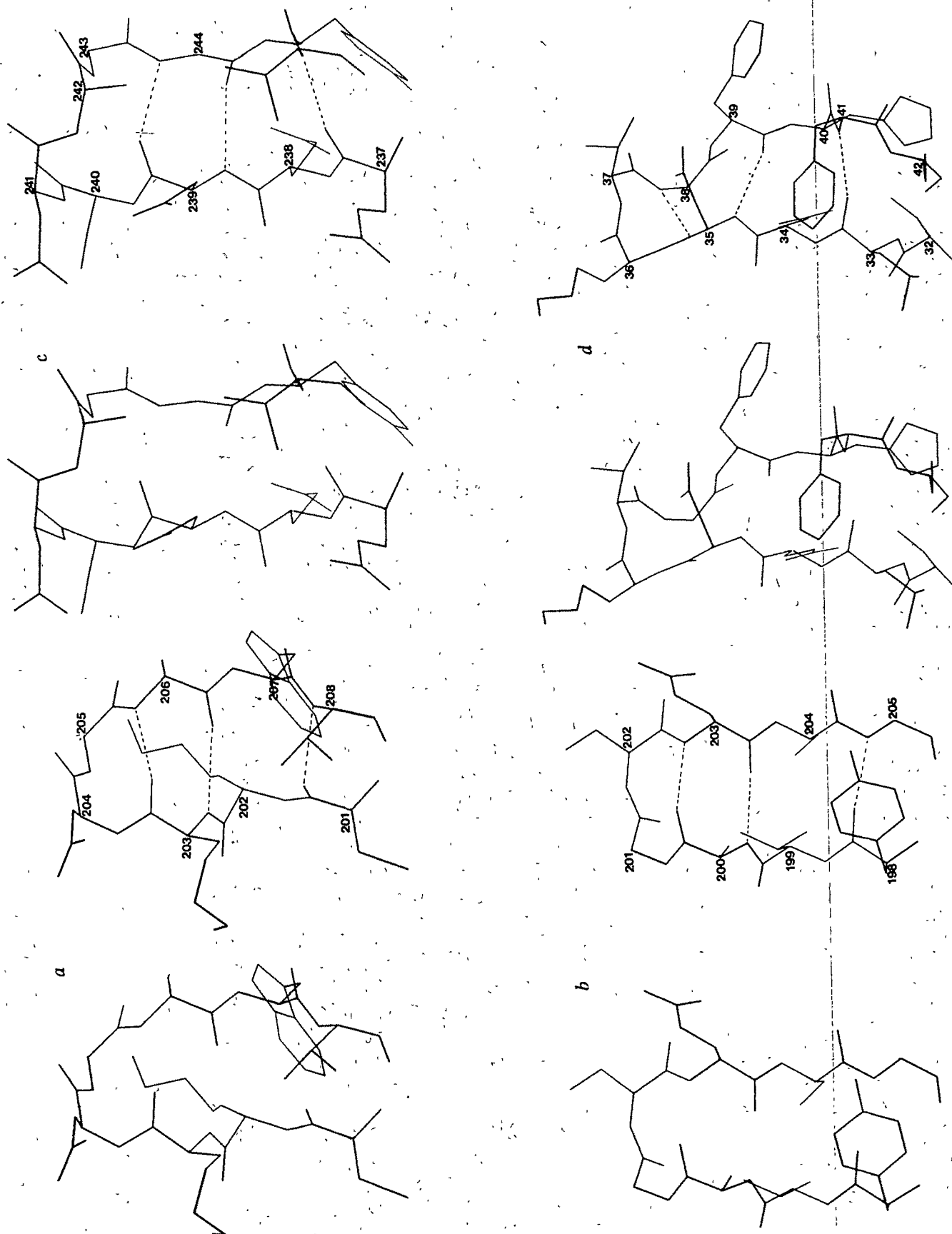


Fig. 3 Stereo diagrams of representative structures for the two-, four- and five-residue hairpin-loop families. *a*, Two-residue hairpin loop with a type I' β -turn; γ -chymotrypsin A, residues 201–208. *b*, Two-residue hairpin loop with a type II' β -turn; penicillopepsin, residues 198–205. *c*, Four-residue hairpin loop with a type I β -turn; penicillopepsin, residues 237–246. Note how the amide NH groups of residues L2, L3 and L4 point inwards towards the centre of the turn and to the carbonyl oxygen of the residue immediately before the loop (–B1). The side chain of this residue may help to neutralize the cluster of NH groups. Some of these bends have been described previously by Isogai *et al.*¹⁷ and Richardson¹⁶. *d*, Five-residue hairpin loop with a type I β -turn and a G1 β -bulge; γ -chymotrypsin A, residues 32–42. This structure is reminiscent of the four-residue family in that the nitrogens of residues L3, L4 and L5 all point inwards towards the carbonyl of residue L1, and an aspartyl oxygen is often observed nearby.

Table 1 β -Hairpin loops in distinct structural families (A) and miscellaneous structures (B)

A								
β -Hairpins with two-residue loops								
Protein	Type I'		Type II'		β -Hairpins with four-residue loops		β -Hairpins with five-residue loops	
	Residue nos	Sequence	Residue nos	Sequence	Residue nos	Sequence	Residue nos	Sequence
Actinidin	170-177	GTEGGVDY						
Superoxide dismutase	21-28	EAKGDTV						
Tosyl elastase	201-208	CLVNGQYA	35-39	YRSGSSWA				
Cytochrome b_5	23-30	LILHYKVY						
Staphylococcal nuclease	92-99	IYADGKMV						
D-Glyceraldehyde 3'-phosphate dehydrogenase	64-71	LVVDEGKKI	299-306	QLSKTFVK				
Immunoglobulin Fab NEW (light and heavy chain)	58-65	KMEDGALV						
Immunoglobulin REI			L64-L71	SKSGSSAT	H202-H211	VNHKPSNTKV	H49-H59*	GYVFIYHGTSDT
Liver alcohol dehydrogenase			L89-L98	SYDRSLRV				
Penicillopepsin	21-28	VTIGGTTL	130-137	FTCRGKPI				
	90-97	VTVGGVTA	198-205	YTAGSQSG	237-246	QQDSNAGGYV	72-82*	SISYGDGSSAS
	258-265	VSISGYTA						
γ -Chymotrypsin A	201-208	CKKNGAWT					32-42*	SLQDKTGFHFC
S-subtilisin inhibitor	84-99	GVWQGRV						
Phospholipase A ₂	76-83	SCSNNEIT						
α -Lytic protease	66-85	ARIGGAVV	48-51	VTRGATKG				
			118-122	VANGSSFV				
Prealbumin					16-25	VLDVARGSPA	33-43*	FRKAADDTWEP
Strep. Griseus	48-51	VSVNGVAH	199-210	LFAGSTAL				
Protease A								
Lysozyme HEW					51-60	TDYGILQINS		
Lysozyme T ₄							17-27*	IYKDETEGYITI
Papain					164-173*	VGYGPNYILI		
Trypsin inhibitor					22-31	FYNKAGLCQ		
Carboxypeptidase A							38-48*	IGRSYEGRPYI
Thermolysin							9-19*	VGRGVLGDQKN
Ovomucoid (3rd domain)							22-32*	PVCGSDNKTYS
B								
β -Hairpins with two-residue loops								
Protein	Type I		β -Hairpins with four-residue loops		β -Hairpins with five-residue loops		Residue nos	Sequence
	Residue nos	Sequence	Residue nos	Sequence	Residue nos	Sequence		
Cytochrome b_5	15-22	HNNSKSTW						
γ -Chromotrypsin A	46-53	LINENWVV						
α -Lytic protease			120-129	TANYAEGAVR	30-43*	EYSINNASLCS		
Strep. Griseus					90-105*	ARVFPNDRAW		
Protease A					117-123*	RVLYNGSYQD		
Prealbumin	110-117	LLSPYSYS			168-178*	TVNYGSSGIVY		
Neurotoxin B	29-36	WSDFRGTI						
Carbonic anhydrase C			62-71*	ILNDGHAFNV				
FAB			L196-L205*	QVTHEGSTVE	L166-L176*	PSKQSNKYAA		
Staphylococcal nuclease					H172-H182*	AVLQSSGLYSL		
Penicillopepsin			24-33*	KLMYKGQPMT	15-25*	IKAIDGDTVKL		
			275-284	GPSGNGSTCL				
			309-318	VFDSDGPOLG				
Glutathione reductase			422-431	VCANKEEKVV	369-379	TVVFHPPIGT		
Concanavalin A					146-156*	ATTGTDGNLEL		
Thermolysin					21-31	NTTYSTYYLQ		
S-subtilisin inhibitor					32-34	TLTCAPGPSGT		

Sequences for two-, four- and five-residue loops. A, Sequences for those hairpins which form distinct structural families, whose ϕ , ψ values are plotted in Fig. 2. B, Sequences for the loops with miscellaneous structures. The standard one-letter amino-acid code is used. Residue numbers are taken from Cambridge Protein Data Bank Files¹⁵ given in ref. 6, except for penicillopepsin for which the more recent 2APP file was used.

* Loops with a single hydrogen bonds between the first (NH) and last (CO) residues in the loop.

+B3 ... for the C-terminal strand. Thus, a two-residue hairpin is described as (... -B3, -B2, -B1, L1, L2, +B1, +B2, +B3 ...).

The distribution of loop length in these 107 hairpins (Fig. 1) clearly shows the predominance of short loop connections, with 70% of all the observed hairpins having <7 residues in their loop regions. Even more striking is the high number of two-residue loops (29 out of 107) and to a lesser extent the four- and five-residue loops (13 and 19 observed, respectively).

The short loop β -hairpins (two-, four- and five-residue) were analysed further by plotting the main-chain dihedral angles ϕ and ψ for these loops (Fig. 2). The plots show that many of the β -hairpins fall into structural families (Table 1a), with the loop regions folded in specific ways. The two-residue β -hairpins all include one of the classic β -turn conformations categorized as types I-VIII³ (see Figs 2b, 3a, b). In a study on all the turns

found in proteins of known structure, Chou and Fasman² found that 60% were type I+III and 15% were type II (Fig. 2a). The mirror images of these two types were rarely observed, type I' constituting only 3% and type II' 5%. For hairpins, however, the types I' and II' turns predominate. Out of the 29 hairpins, 15 were type I', 10 type II' and only 4 type I. The total absence of type II turns in the hairpins and relative lack of type I turns strongly implies that these turns, which are by far the most commonly observed throughout proteins, are not compatible with an antiparallel β -hairpin. In contrast, their mirror images, which are thought to be energetically less favourable because of steric hindrance³, are clearly preferred in the context of a β -hairpin. Preliminary studies suggest that the type I' turn has the correct twist to match the relative twist which is always observed between adjacent strands⁷ (Fig. 3a). The mirror-image

type I has the incorrect twist and may therefore be unfavourable. In contrast, the types II and II' turns are relatively planar (see Fig. 3b). However, model-building two β -strands with an average twist and standard β -turns on a computer graphics system shows clearly that the types I' and II' turns give acceptable hairpins, whilst the strands rapidly diverge when a type I or II turn is included.

The sequences of the two-residue hairpins are shown in Table 1. As might have been expected from the observed ϕ, ψ plot, 12 of the 15 type I' hairpins have glycine at position L2, while position L1 is dominated by glycine, asparagine and aspartic acid (left-handed helical residues⁸). Similarly, 7 of the 10 type II' hairpins have glycine at position L1, whilst position L2 is usually the polar serine or threonine. The absence of proline is striking.

The 13 four-residue loop hairpins include various turn conformations, with the dominant grouping a family of six structures with a type I turn (Figs 2c, 3c). Four of these hairpins have a particularly similar conformation, with residues L1, L2 and L3 in the α -helical portion of the ϕ, ψ plot and residue L4 (glycine or asparagine) in the $+/+$ quadrant. The tip of these four-residue hairpins is bent over, rather like an erect hooded cobra.

The ϕ, ψ plots for the 19 five-residue loop hairpins showed clearly that one group including eight examples all adopted the same structure (Figs 2d, 3d). This may be described as a type I turn, with a hydrogen bond between the carbonyl residue L1 and the amide of residue L4, and a G1 type β -bulge⁹. This structure demands a glycine at position L4, which is observed in six of the eight sequences, with asparagine and aspartic acid in the other two (Table 1). Of the remaining five-residue loops, three include an L2(CO)-L5(NH) hydrogen bond (although the ϕ, ψ values are different) and four others have glycine at position L4, although they show no structural similarity.

One of the major aims of our study was to search for patterns to aid the prediction of hairpins from sequence. The two-residue loops are strongly selective for amino-acid type. For a type I' turn, L1 = Asn, Asp, Gly; L2 = Gly; for a type II' turn, L1 = Gly and L2 = Ser, Thr or polar. The four- and five-residue loop families have a weaker preference for glycine at position L4. The other residues are usually hydrophilic, as expected for an exposed loop, and often include the classical turn-forming residues.

There is also evidence for distinctive patterns in the residues on either side of the hairpin loop. In addition to the usual alternation of hydrophobic and hydrophilic residues, a matching of residue types across the hairpin is observed. Thus, residues +B3 and -B3 are usually both hydrophobic or both hydrophilic (80% of sequences in Table 1), similarly residues +B2 and -B2 (67%). Such paired residues point in the same direction and are usually therefore in a very similar environment.

These patterns are being incorporated into our template prediction algorithm, which aims to recognize β -hairpins rather than just β -strands. Currently, for the β family of proteins, we

obtain an ~8% improvement in prediction accuracy over the conventional secondary structure prediction algorithms. This is similar to the improvement obtained for the $\beta\alpha\beta$ proteins⁵.

Our results show that the irregular coil regions connecting secondary structures in proteins may include structural and sequence patterns. This limited survey of β -hairpins indicates that the short loop hairpins are highly selective in sequence and can provide a valuable aid to *ab initio* prediction and model-building studies. Insertions and deletions most commonly occur in the loop regions of proteins¹⁰, so that prediction of an unknown structure by homology to a known structure becomes a problem of building the loop regions (for example, renin¹¹). We are currently studying the longer loops and homologous β -hairpins to determine the effects on loop conformation.

In the β -sandwich structures there are essentially only two types of connections between β -strands—a β -hairpin between adjacent H-bonded strands and β -arch between strands on opposite sheets crossing the top of the sandwich. In terms of chain topology, it is critical to differentiate a β -arch from a β -hairpin. Although this will clearly depend largely on the amino-acid composition of the strands (for example, very hydrophobic strands will occupy central positions in the sheet¹²), the sequence of the connecting loop may also be influential. Preliminary studies of β -arches show that their length distribution is grossly different from that of β -hairpins, with a peak for the eight-residue loop. Sequence patterns may also be found for connections between helices^{13,14} and between strands and helices in the $\beta\alpha\beta$ unit. With such information on the loop regions of proteins, the prediction of structure from sequence by template and pattern recognition methods becomes very attractive.

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The Medical Renaissance of the Sixteenth Century. A. WEAR et al. (eds). Cambridge University Press. 1984. Pp.349. ISBN 0-521-30112-3. Pp.

Microbiological Methods for Environmental Biotechnology. J. M. GRAINGER and J. M. LYNCH (eds). Academic. 1984. Pp.421. ISBN 0-12-295040-2. \$65, £45.

Microspheres and Drug Therapy: Pharmaceutical, Immunological and Medical Aspects. S. S. DAVID et al. (eds). Elsevier. 1984. Pp.448. ISBN 0-444-80577-X. \$109.50, Dfl. 285.

Monoamine Oxidase and Disease: Prospects for Therapy with Reversible Inhibitors. K. F. TIPTON, P. DOSTERT and M. STROLIN BENEDETTI (eds). Academic. 1984. Pp.694. ISBN 0-12-691660-8. \$44.50, £34.50.

Monoclonal and Anti-Idiotypic Antibodies: Probes for Receptor Structure and Function. J. CRAIG VENTER, CLAIRE M. FRASER and JON LINDSTROM (eds). Alan R. Liss. 1984. Pp.194. ISBN 0-8451-3703-4. £52.

Neurotransmitters, Seizures and Epilepsy. RUGGERO G. FARIELLA et al. (eds). Raven. 1984. Pp.391. ISBN 0-88167-057-X. \$68.

Novel Approaches to Cancer Chemotherapy. PRASAD S. SUNKARA (ed.). Academic. 1984. Pp.383. ISBN 0-12-676980-X. \$65, £50.

Nutritional Prevention of Cardiovascular Disease. WALTER LOVENBURG and YUKIO YAMORI (eds). Academic. 1984. Pp.410. ISBN 0-12-456010-5. \$38, £29.50.

Organ Based Autoimmune Diseases. J. M. CRUSE and R. E. LEWIS (eds). Karger. 1985. Pp.278. ISBN 3-8055-3929-0. DM 178, \$89.25.

Pharmacology of Intestinal Permeation II. T. Z. CSÁKY (ed.). Springer-Verlag. 1984. Pp.589. ISBN 3-540-13101-9/0-387-13101-9. \$188.20.

Physiological Genetics of Agricultural Crops. By ANDOR BALINT. Akadémiai Kiadó, Budapest. 1984. Pp.167. ISBN 963-05-3288-3. £10.50.

Principles of Health Risk Assessment. By PAOLO F. RICCI. Prentice-Hall. 1985. Pp.417. ISBN 0-13-709197-4. Pp.

Progress in Nucleic Acid Research and Molecular Biology, Vol. 31. WALDO E. COHN and KIVIE MOLDAVE (eds). Academic. 1984. Pp.484. ISBN 0-12-540031-4. \$58, £43.50.

Prostacyclin: Clinical Trials. RICHARD J. GRYGLEWSKI, ANDREW SZCZĘKLIK and JOHN C. McGIFF (eds). Raven. 1984. Pp.159. ISBN 0-88167-051-0. \$41.

Recent Advances in Systemic Lupus Erythematosus. P. H. LAMBERT, L. PERRIN and S. IZUI (eds). Academic. 1984. Pp.359. ISBN 0-12-434620-0. \$35, £24.

Reviews in Perinatal Medicine, Vol. 5. EMILE M. SCARPELLI and ERMELANDO V. COSMI (eds). Alan R. Liss. 1984. Pp.214. ISBN 0-8451-0400-4. £32.

Tissue Culture of Epithelial Cells. MARY TAUB (ed.). Plenum. 1984. Pp.288. ISBN 0-306-41740-5. Pp.

Vasodilator Mechanism, Vol. 38. P. M. VANHOUTTE and S. F. VATNER (eds). Karger. 1983. Pp.284. ISBN 3-8055-3903-7. SwFr. 138, DM 165.

August meeting

21–23 August 1985, **International Workshop on Molecular Biosciences and Bio technology**, Madras, India (Convenors of the workshop, SPIC Main Office, 97 Mount Road, Madras-600 032, India).

September meetings

15–20 September 1985, **International Symposium on Renal Basement Membranes**, London, UK (Dr R. G. Price, Biochemistry Dept., Queen Elizabeth College, Campden Hill Road, London, W8 7AH, UK).

16–20 September 1985, **International Basement Tectonics Conference**, Santa Fe, New Mexico, USA (M. Aldrich, General Chairman, Mail Stop D462, Los Alamos National Laboratory, Los Alamos, New Mexico 87545, USA)

November meeting

21–22 November 1985, **First International Conference on Protein Engineering**, London, UK (Miss H. Raquet, Oyez Scientific and Technical Services Ltd, Bath House, 3rd Floor, 56 Holborn Viaduct, London, UK).

Appointments

Dr Robert Sharpe has been appointed director of the Lord Dowding Fund for Humane Research, in succession to the late Bernard Conyers. The fund actively promotes the development of viable alternative techniques to the use of living animals for research and testing.

Professor John L. Jinks, Vice-Principal of Birmingham University, has been appointed to the board of directors of the Agricultural Genetics Co. Ltd (AGC), the Cambridge-based biotechnology company.

Four new members have joined the Imperial Cancer Research Fund Council. They are **Professor Robert Mahler**, Consultant Physician at Northwick Park Hospital; **Sir John Vane**, Group Research and Development Director, The Wellcome Foundation; **Professor John Evans**, Director of the MRC's Clinical Population Cytogenetics Unit; and **Professor David Weatherall**, Nuffield Professor of Clinical Medicine at the University of Oxford.

Dr J. W. Almond, lecturer in microbiology at the University of Leicester, has been appointed Professor of microbiology at Reading University.

Reading University has appointed **Dr D. L. Pyle** as Professor of biotechnology. He is currently at the Imperial College of Science and Technology, University of London.

The Heriot-Watt University has announced two new professorships. **James L. Murray** has been appointed to a new Chair of Computer-Aided Engineering in the department of mechanical engineering—with effect from 1 May 1985, and **John A. Swaffield** has been appointed to a new Chair of Building Services Engineering in the department of building—from 1 September 1985.

Dr P. G. Hare, at present reader in economics at the University of Stirling, has been appointed Professor of economics at Heriot-Watt University, Edinburgh.

Raymond E. Wetton, Chairman of Polymer Laboratories Ltd, has been appointed Visiting Professor in the department of chemistry at Loughborough University.

Richard K. Root, Professor and vice-chairman of the department of medicine at the University of Washington, has been named the new chairman of the University of California, San Francisco Department of Medicine.

The following have been elected to the Fellowship of Imperial College, London: **Emeritus Professor J.H. Argyris**, Imperial College and University of Stuttgart; **Mr John Egan**, Chairman and Chief Executive, Jaguar Cars Ltd; **The Rt Hon Lord Carr of Hadley**, Chairman, Prudential Corporation and Prudential Assurance Company, Governor, Imperial College; **Mr H.M. Neal**, Chairman, City and Guilds of London Institute; **Professor Sir Randolph Quirk**, Vice-Chancellor, University of London; **Emeritus Professor J. Sutton**, Senior Research Fellow, Centre for Environmental Technology at Imperial College.

Awards

The Simon-Fraser University R. H. Wright award in Olfactory Research has been made to **Professor Karl-Ernst Kaissling** of the University of Seewiesen, FRG. He was cited for his pioneering work in the understanding of the process of olfaction (the sense of smell).

The Royal Society of Chemistry and the Society of Maccabaeans have named the joint winners of this year's Meldola Medal and Prize. **Dr James Darwent**, lecturer in chemistry at Birkbeck College, London, and **Dr Ian Rothwell**, assistant Professor at Purdue University, Indiana, USA, will each receive a bronze medal and £100. The award is made annually to the most promising British chemist under the age of 30.

Dr Mary F. Lyon, FRS, Deputy Director and Head of Genetics Division at the MRC Radiobiology Unit in Oxfordshire has been awarded the 4th Sanremo International Prize for genetic research.

A multidisciplinary team led by **Professor Ronald J. Roberts** at the Institute of Aquaculture, University of Stirling, UK, has been nominated by the British Government for this year's UNESCO Science prize. The nomination recognizes the work of the team on the development of an intensive culture system for farming Tilapia. This is the most important farmed fish of Africa and South-East Asia, which is making an increasing contribution to protein availability in developing countries.

The North Jersey Section of the American Chemical Society has announced that **Alexandra Pines**, Professor of Chemistry at the University of California, Berkeley, has been selected to receive the 1985 Leo Hendrick Baekeland Award. The award, sponsored by Union Carbide Corporation, is made in recognition of his work in the field of magnetic resonance.

Applications are invited for the MRC Senior Fellowships (nonclinical), to arrive before 30 September 1985. The scheme is intended to provide support for a small number of outstanding scientists, and covers personal support for initially the first six years, with provision for supporting staff/expenses/equipment for the first three years. Applicants should be untenured, but with no less than three years' postdoctoral research experience at the date on which they would take up their fellowship. Applicants should be between the ages of 26 and 32 (inclusive) on 30 September 1985, and can obtain further information from the Grants Section at MRC's Headquarters Office (MRC, 20 Park Crescent, London W1N 4AL, UK).

The International Union Against Cancer will award "International Cancer Research Technology Transfer" grants for research on cancer. The purpose of the programme is to promote the transfer of information about new or improved techniques or methods in areas of basic, clinical or behavioural research, in order to further the progress of cancer research. The available funds will permit researchers of any nationality to visit a research centre, or centres, abroad for a period not exceeding 28 days. Information and application forms can be obtained from: International Union against Cancer, Rue du Conseil-General 3, 1205 Geneva, Switzerland.

Miscellaneous

The Voluntary Licensing Authority (VLA) for human *in vitro* fertilization (IVF) has been set up by the Medical Research Council and the Royal College of Obstetricians and Gynaecologists. Centres offering an IVF service or undertaking research in this field may apply to the authority for approval of their work.

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UNIVERSITY COLLEGE CARDIFF

DEPARTMENT OF BIOCHEMISTRY

TUTORIAL FELLOW

Applications are invited from graduates for the above post. Salary range £6,600 — £7,055 per annum. The post is for three years commencing in October. The appointee will have a First or Upper 2nd Class Honours Degree and will be permitted to register for a PhD. There will be some limited teaching duties and the appointee will join Professor K.S. Dodgson's research group in studies on the biodegradation of surfactants and other industrially important compounds.

Applications (2 copies), together with the names and addresses of two referees should be forwarded to the Establishment Officer, University College, PO Box 78, Cardiff, CF1 1X, from whom further particulars are available. Closing date 31st July, 1985. Ref: 2978. (7062)A

THE UNIVERSITY OF BRISTOL

DEPARTMENT OF
VETERINARY MEDICINE

Applications are invited for a POSTGRADUATE RESEARCH ASSISTANT (Range 1B)

to work on the Immunology of the gastrointestinal tract of horses. The successful candidate will join a multi-disciplinary group investigating responses to microbial and dietary antigens and would be expected to register for a higher degree. The post funded for three years by the Horse-race Betting Levy Board is open to veterinary and biological science graduates. Starting salary: up to £7,980 per annum.

Applications should be sent enclosing a curriculum vitae and details of two referees to Professor F J Bourne, Department of Veterinary Medicine, Langford House, Langford Bristol, BS18 7DU, by 22 July 1985. (7061)A

UNIVERSITY OF NOTTINGHAM MEDICAL SCHOOL

DEPARTMENT OF BIOCHEMISTRY
POST-DOCTORAL FELLOW

Applications are invited for a research fellowship financed by the British Heart Foundation for a study of phosphoinositide metabolism and the molecular mechanisms mediating the responses of cardiac muscarinic receptors. The fellowship is tenable for two years initially from 1st October, 1985 at a starting salary of £7,980 or £8,450 depending on age and experience. Membership of USS included

Applicants should send curriculum vitae and the names of two referees to Professor J N Hawthorne, Department of Biochemistry, Medical School, Queen's Medical Centre, Nottingham NG7 2UH, from whom further details may be obtained (telephone: 0602-700111 Ext 3488). Closing date 31st August, 1985 (7065)A

UNIVERSITY OF LIVERPOOL

DEPARTMENTS OF IMMUNOLOGY
AND BIOCHEMISTRY

Applications are invited for the post of Senior Research Assistant in the Department of Biochemistry to work on an investigation into gene cloning of human oncotrophoblast antigens defined by monoclonal antibodies. This work has important implications for our understanding of materno-foetal interactions as well as the process of oncogenesis. This is a post-doctoral position for which experience in recombinant DNA techniques would be a strong advantage.

Initial salary within the range £7,520 — £8,920 per annum

Applications, together with the names of three referees, should be received not later than 26 July 1985 by the Registrar, The University, PO Box 147, Liverpool, L69 3BX, from whom further particulars may be obtained. Quote Ref: RV/14 (7074)A

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In addition to a very competitive salary we offer excellent conditions of employment, good technical and recreational facilities and assistance with relocation where appropriate.

Applications enclosing a detailed CV should be addressed to:



Miss J E Huckvale, Personnel Officer,
Imperial Chemical Industries plc,
Pharmaceuticals Division,
Mereside, Alderley Park,
Macclesfield, Cheshire. SK10 4TG.



INTERNATIONAL CROPS RESEARCH INSTITUTE FOR THE SEMI-ARID TROPICS

needs

A Groundnut Breeder at Niamey, Niger

To initiate and develop a regional groundnut breeding program in West Africa in conjunction with a groundnut pathologist and as part of a multidisciplinary regional team whose objective is to develop high-yielding stable varieties of groundnut suitable for use in farming systems of the region and to develop materials with resistances to the main biotic and abiotic constraints presently limited production, in particular drought, leaf spots, and rosette.

The Groundnut Breeder will conduct in cooperation with national programs of the region, multilocal yield trials and nurseries and supply cooperators with breeding materials; work with national programs to develop their effective capability for the improvement of groundnuts including periodic training programs; contribute to the development of a regional groundnut research network, and evaluate and maintain germplasm both local and exotic for desirable characters. Cooperate closely with the ICRISAT Center program and ICRISAT's regional program in southern African, in multilocal evaluation and selection to develop widely adapted parental material. A Ph.D. in plant breeding with five years experience in breeding groundnuts or related crops, ability to work with national and international scientists from a range of disciplines, and to travel frequently to conduct research in the groundnut growing areas of West Africa are essential. He should have excellent command of English. Working knowledge of French and experience in tropical agricultural research particularly in semi-arid environments are desirable.

A Groundnut Pathologist at Niamey, Niger

To work as part of a multidisciplinary regional team to develop for the limited-resource farmer high-yielding and stable varieties of groundnut, to insure that such varieties carry adequate epidemics, and to conduct research that will contribute to enhanced knowledge and understanding of groundnut diseases in the region. The pathologist will develop a research program on diseases of groundnut, both fungal and viral, in West Africa in cooperation with a groundnut breeder and other disciplines and will cooperate in screening materials and in the establishment of regional testing networks and training programs. A Ph.D. in plant pathology with several years' experience in the field of host plant resistance, ability to work with national and international scientists from a range of disciplines, and to travel frequently to conduct research in the groundnut growing areas of West Africa are essential. He should have excellent command of English. A working knowledge of French, and experience in tropical agricultural research particulars in a semi-arid environment are desirable.

The positions are on a 3-year contractual basis with possibilities of further extension. The posts are located at the ICRISAT Sahelian Center near Niamey, Niger, which is the regional headquarters for ICRISAT's West African programs. Niamey, the capital city, has good living conditions, international airline connections, and educational facilities for children in French and English.

Send résumé and names and addresses of three referees within 60 days of the date of the advertisement to the Director General, ICRISAT, Patancheru P.O., Andhra Pradesh 502 324, India.

(W2012)A

PHYSICIST:

To design, test and calibrate at atmospheric sounder and analyze data produced. Must be familiar with experimental and theoretical techniques of pressure modulation radiometry. Four years experience. PhD in physics or astronomy \$2,500 — \$3,000/month. Pasadena area. Send this ad and your résumé to: Job Order # 2943, P.O. Box 865, Sacramento, CA 95804 not later than July 29, 1985. (NW23)A

Biochemist

with experience in DNA sequence analysis and allied topics required for novel enterprise. Give full details of past experience.

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c/o Nature
4 Little Essex Street
London WC2R 3LF

(7107)A

THE UNIVERSITY OF BERGEN

invites applications for a vacant chair in

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Salary is on the scale 33, Nkr 233,752, p.a. gross, of which Nkr 4,010 p.a. are paid in pension contributions.

The chair will be appointed on the understanding that any changes in scientific duties, pension or retiring age made by law or by the King with the agreement of Parliament are to be accepted without compensation.

Applicants should submit 5 copies of scientific work — published or unpublished — which they wish to be considered for the appointment as well as 6 copies of a list of all scientific contributions with information on where they were published. Scientific contributions are to be submitted, in numbered order an in 5 groups, to the science faculty of the University of Bergen within one month of the closing date for applications. Scientific manuscripts in preparation may be submitted within 3 months of the closing date for applications provided notice of intent is given on submitting the other publications. Applicants are otherwise referred to the current rules for the procedure to be followed in the appointment of professorships and readerships. A résumé of the vacant chair can be obtained on request from: Sekretariatet for Det matematisk-naturvitenskapelige fakultet, Postboks 25, 5014 Bergen-Universitetet, Norway.

Applications, which must include a complete curriculum vitae, should be addressed to the King and be sent together with relevant certificates and one copy of a list of publications to Det matematisk-naturvitenskapelige fakultet, Postboks 25, 5014 Bergen-Universitetet, within Oct, 1985. (W2020)A

BILLERICAY BURNS RESEARCH TRUST SENIOR RESEARCH FELLOW

Applications are invited for a Senior Research Fellow for a project in burns research. Considerable experience in independent research will be required. The Fellow will be expected to pursue not only his own project but also to give advice to guidance on their research projects to surgical staff at this Regional Centre for Plastic Surgery and Burns.

The Fellow's research project might be in the structural, biochemical, pharmacological, physiological or experimental pathological fields.

It is envisaged that the Fellow would be of a status such as would enable the Trust to seek for him or her an honorary university appointment in one of the University of London Medical Schools.

Further details may be obtained from:

A. F. Wallace, F.R.C.S.
N.E. Thames Regional Plastic Surgery Centre,
St. Andrew's Hospital,
Billericay,
Essex.

(7082)A

THE DEPARTMENT OF PHYSIOLOGY University of Manitoba

invites applications from young investigators in the field of

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to be appointed at the level of Assistant Professor

The successful candidates will be expected to compete for local and national scholarship awards and develop their independent research programs. Applicants with backgrounds in membrane transport, electrophysiology, molecular biology or hemodynamics are encouraged to submit their curriculum vitae, an outline of their research interests as well as to arrange for three letters of reference to be sent to Dr. Henry G. Friesen, Professor and Head, Department of Physiology, Faculty of Medicine, University of Manitoba, Winnipeg, Manitoba, R3E 0W3 Canada. Deadline for application is 2 September 1985. The University of Manitoba is an Equal Opportunity/Affirmative Employer, but in accordance with Canadian Immigration, priority will be given to Canadian citizens and permanent residents of Canada. (NW12)A

INTERNATIONAL INSTITUTE FOR AEROSPACE SURVEY AND EARTH SCIENCES



The ITC is concerned with international scientific education, research and consulting in aerial photography and teledetection, photogrammetry, aerospace survey application in land resources studies, agriculture, earth sciences, human settlements, geo-data processing and cartography, in particular directed towards the needs of developing countries.

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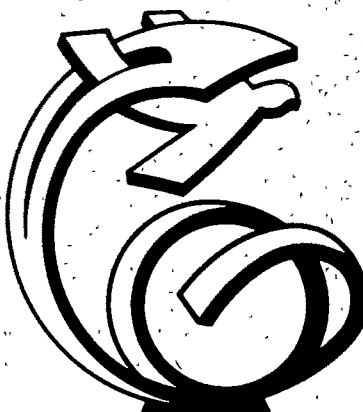
- teaching at the post-graduate level, particularly in a new one year diploma course in LIS
- initiating and undertaking research
- coordinating the research activities of an inter-disciplinary group in geo-information systems
- acting as a consultant to projects.

Some foreign travel can be expected.

The Institute is placing increasing emphasis on the development of geo-based and land information systems. It is supported by excellent facilities in aerial photography, photogrammetry, remote sensing, image processing, cartography, natural resource inventories and human settlement analysis. Currently these include aircraft, analytical plotters, image processing and interactive graphic systems, scanners and plotting devices.

GIS/LIS an area of growth in this Institute. The person appointed will be expected to play a key role, and can anticipate a rewarding career.

For further information make early contact with the rector of the Institute, Prof.dr.ir. K.J. Beek, if preferred by telephone; formal applications should be addressed to the Head of Personnel & Organisation.



350 Boulevard.1945,
P.O. Box 6
7500 AA Enschede
The Netherlands.
Phone 903153-320330.

In addition to this particular post, the Institute is always interested in receiving curriculum vitae's from persons, within the fields mentioned above who are available for both short and long term appointments in the Netherlands and/or abroad.



GUINNESS

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The excellent benefits package provided will include a highly competitive salary, non-contributory pension, profit share, free lunch and medical benefits. Starting point will be related to previous experience and qualifications.

Applications containing CV details should be sent to

Dr. A. J. Forage
Manager, Guinness Research Centre
St. James's Gate
Dublin 8 Ireland.

by 26 July 1985.

(7102)A

BIOCHEMIST/ MOLECULAR BIOLOGIST

The Department of Biochemistry and Biophysics is seeking applications for two Assistant or Associate Professorships. These are 12 month, tenure track positions jointly funded by the College of Agriculture and the Texas Agricultural Experiment Station. Outstanding individuals having research interests dealing with the molecular aspects of regulation, structure, mechanism or function in microbial, animal or plant systems are sought. In addition to research, participation in the department graduate and undergraduate teaching programs will be required. A terminal degree in either biochemistry or a related field plus appropriate postdoctoral experience is required.

Applicants should send a curriculum vitae, list of publications, a brief description of current and future research directions, and names of three references to:

Dr. E. G. Sander, Head
Department of Biochemistry and Biophysics
Texas A&M University
College Station, TX 77840-2128

Applications will be accepted until October 15, 1985, or until suitable candidates are found.

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(NW76)A

THE IRISH EQUINE CENTRE DIRECTOR/ SCIENTIFIC DIRECTOR

The Irish Equine Foundation Limited invites applications for the post of Director/Scientific Director of the Irish Equine Centre. The Irish Equine Centre is located at Johnstown, Naas, County Kildare, Ireland and is engaged in the investigation, diagnosis and research into equine diseases. Phase 1 in the development of the Centre includes departments of pathology, bacteriology, virology and immuno-haematology.

Applicants should have a registrable veterinary qualification. The successful candidate will have a doctorate or its equivalent, a distinguished research career and promise of administrative skills to provide leadership and co-ordination of a diverse group.

Salary, which will not be less than IR£35,000 per annum, and other benefits will be commensurate with the responsibilities of the position and dependent on experience.

Closing date for applications is September 20th 1985.

Applicants should include a full Curriculum Vitae and the names and addresses of two referees who will be contacted only with the prior permission of the applicant.

On behalf of the Board of Governors, Irish Equine Centre, P.W. McGrath, LL.D., Chairman.

Applications should be submitted to the Secretary, The Irish Equine Foundation Limited, Irish Equine Centre, Johnstown, Naas, Co. Kildare, Ireland. (7075)A

MERRELL DOW RESEARCH INSTITUTE

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We are part of the Merrell Dow Research Institute. Our Center located in Strasbourg is dedicated to apply basic research to discover new therapeutic leads. We are looking for two

BIOCHEMISTS

(Ph.D. + 1 or 2 year postdoctoral experience at least) to join our multidisciplinary research team.

The first position requires interest and proven skill in all aspects of enzymology (purification, assay, modification, etc.).

The second position is offered to a young biochemist with a strong background in organic chemistry, more oriented towards mechanistic approaches.

Essential personal qualities are initiative, creativity and ability to collaborate with other scientists within our team.

If you are interested in these positions, please send C.V. with names of 3 referees we may contact to:

M. J. Bildstein
Personnel Department
Merrell Dow Research Institute
16 rue d'Ankara
67084 Strasbourg Cedex.

(W2031)A

UNIVERSITY OF OXFORD
Department of Biochemistry
POST-GRADUATE
RESEARCH ASSISTANT —
1B

(Ref: BC/131)

A Research Assistant Grade 1B is required to work in the Eukaryotic Gene Expression Group of Drs. A.J. and S.M. Kingsman. Experience in microbial genetics or recombinant DNA and/or mammalian cell culture procedures would be an advantage.

The appointment is for three years and will be made on the Scale £6,600 — £10,330 p.a., depending upon age and qualifications.

Applications, including the names and addresses of two referees should be sent, by 31st July, 1985 to:

**The Administrator,
 Department of Biochemistry,
 South Parks Road,
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Graduate programs leading to Ph.D. or M.S. are available. Stipends: \$15,996 to \$30,000, depending upon exercise.

Send curriculum vitae, three references and statement of research interests to: Dr Kenneth Isutsu, Department of Oral Biology SB-22, University of Washington, Seattle, Wash. 98195 at earliest possible date.

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(NW7)A

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MOLECULAR BIOLOGIST

To participate in projects aimed at determining the role of oncogenes in human cancer using cDNA cloning, *in situ* hybridization and bacterial expression of antigens. Ph.D. in Molecular Biology, although candidates with Masters degree and appropriate experience will be considered.

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 Oncogene Science Inc., 222 Station Plaza North, Suite 330,
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(NW31)A

PATHOLOGIST

Experimental and/or Comparative

Requirements: M.D., D.V.M., or Ph.D. in Pathology Board Certified or eligible.

Duties: Research and diagnostic service in a comprehensive pathology laboratory in a large, modern research facility. Independent research in one or more areas of interest, as well as collaborative studies with departmental research staff. Diagnostic service will involve human and animal pathology if applicant holds an M.D. Facilities for morphologic studies are excellent, including quantitative light microscopy, TEM, SEM and high-voltage electron microscopy. The Wadsworth Center has ongoing programs in every area of basic and applied biomedical science, clinical and environmental.

Salary: Open, dependent on degrees, certification held and experience.

Send résumé to: Doris N. Collins, M.D., Wadsworth Center for Laboratories and Research, New York State Department of Health, Empire State Plaza, Albany, NY 12201. (NW17)A

Colaiste na hOllscoile Corcaigh

UNIVERSITY COLLEGE CORK

BIOCHEMISTRY DEPARTMENT

Applications are invited for a full-time temporary appointment at the level of Assistant Lecturer or College Lecturer in the Department of Biochemistry. The appointment, which will commence on 1 October, 1985, will be for one year in the first instance with the possibility of extension for two further years. Applicants should preferably have research experience in protein or nuclear acid biochemistry, but candidates with research experience in other fields will be considered. The salary will be within the range IR£10,432 to IR£14,179 depending on experience.

Candidates should make a written application, including the names of two referees, to reach the undersigned not later than Friday, 16 August, 1985. Professor S. Doonan, Head of Biochemistry Department, University College, Cork, Ireland.

(7077)A

Ministry of Agriculture,
Fisheries and Food
Food Science Division, London

Chemist

Working in a small group you will provide scientific advice on all aspects of the manufacture, composition and control of materials intended to come into contact with food. You will be involved in reviewing the possibility of contamination of food supply by chemicals arising from packaging materials and with the design, commissioning and supervision of research projects concerning these materials.

Appointment as Senior Scientific Officer. Ref: SB/70/AE.

Fisheries Research Directorate,
Burnham-on-Crouch

Fisheries Biologist

This is an opportunity to study the effects of biocides on freshwater and estuarine organisms and to assess the effects of other chemicals on aquatic life. You will set up and conduct tests and analyse the data obtained. A commitment to field work and duty at sea is essential.

Appointment as Scientific Officer. Ref: SB/96/AD.

Salary and Qualifications

Senior Scientific Officer: £11,135-£14,015 (includes £1365 Inner London Weighting), 1st or 2nd class honours degree or equivalent in chemistry or related discipline and at least 4 years' relevant postgraduate experience.

Scientific Officer: £6,190-£8,560. An honours degree in biology or biochemistry. A knowledge of eco-toxicology would be advantageous.

For further details and an application form (to be returned by 9 August 1985) write to Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, or telephone Basingstoke (0256) 468551 (answering service operates outside office hours)

Please quote appropriate reference.

The Civil Service is an equal opportunity employer (7113)A

**Scientific
Civil Service**

Plant Cell Biologists

The Agricultural Research Division of American Cyanamid has immediate opportunities for Plant Cell Biologists who will form the nucleus of a program to regenerate from culture crops resistant to our new herbicides.

We seek individuals whose strong background in regeneration of plants from cell and tissue cultures prepares them to play a significant and highly visible role in this project. Your qualifications should include 2-3 years' active and highly productive experience in Post-Doctoral cellular or molecular biology.

In return, we offer a very competitive compensation and benefits package and the opportunity for significant professional growth and advancement. For prompt and confidential consideration, forward your resume, including salary requirements, to: Employment Office



American Cyanamid Company
Agricultural Research Division
PO Box 400
Princeton, New Jersey 08540
An Equal Opportunity Employer M/F/H
No Agencies Please

(NW16)A

THE UNIVERSITY OF NEW SOUTH WALES
Sydney, Australia

INDUSTRIAL LASER PHOTOCHEMISTRY

SENIOR RESEARCH SCIENTIST, RESEARCH SCIENTIST, POSTDOCTORAL

Applications are invited for several positions in a research group which is developing laser-based industrial photochemical processes. Experience in lasers, photochemistry, optical spectroscopy and industrial applications would be helpful.

The University of New South Wales contracted with the Commonwealth of Australia to work intimately with the Australian chemical industry to develop new commercial processes using lasers. Successful candidate processes are to be taken to the pilot plant design stage.

Since July, 1984, funding of A\$1 million has been used to set up a laboratory including four excimer lasers, pulsed dye and CW ring dye lasers, and a Fourier Transform UV-IR Spectrometer. A further A\$1 million has now been contracted to provide for additional staff and equipment. Appointments will be for one year, with possibilities of continuing depending on additional funding. Sabbatical or postdoctoral applicants are welcome.

Work involves investigation of scientific feasibility of proposed processes, optimization studies, small-scale reactor tests, followed by preparation of estimates for prototype production plants. Already one pilot plant design has been submitted to the government for additional funding and a second pilot plant is under discussion with the company involved. Funding is available for patents, and two worldwide patents have been submitted.

In addition to working with state-of-the-art equipment and collaborating with industry on real-world problems, applicants will have the opportunity of working with a Project Technical Leader of international stature. Industrial liaison is handled by the Project Manager. Project staff will collaborate with workers from industry who are associated with the six processes being investigated. Applicants will be expected to be technically strong, and opportunities exist for publication in the open literature.

Salaries will be commensurate with experience, and will be up to A\$38,700. Applications close September 15. Appointees will be expected to commence work as soon as possible, and no later than January 1, 1986.

Call Dr George Paul, Project Manager (612) 697-4586, (612) 419-5865 (home). Telex UNREG AA26054. Or write Dr. Paul, School of Physics, University of New South Wales, Kensington, NSW 2033, Australia.

EQUALITY OF EMPLOYMENT OPPORTUNITY IS UNIVERSITY POLICY.
(W2032)A

FACULTY POSITIONS IN ELECTRICAL ENGINEERING (SOLID STATE DEVICE AND MICROELECTRONICS AREA)

The Pennsylvania State University at University Park, Pennsylvania invites applications for tenure track faculty positions at the Assistant Associate and Full Professor ranks in Electrical Engineering in the area of Solid State Device and Microelectronics. Applicants should have an earned doctorate in electrical engineering, engineering physics or related fields, and have demonstrated the ability to conduct research and teaching in the field of Solid State Device and Microelectronics.

The successful candidates are expected to join the existing effort in the solid state device research and help develop it into one of prominence. The College of Engineering has made substantial commitments to support this program by hiring of an endowed professor in this area.

Currently, research in the solid state device and related materials area includes research in silicon device processing, such as plasma and reactive ion etching, thermal nitridation of silicon and various oxide research including ultra-thin oxides, and defect control. In addition, research in III-V device area includes material defects, electrical noise and performance improvement of micron and sub-micron structures.

Combined research in the solid state device area typically amounts to about one million dollars annually. Collaborative research opportunities exist with the Materials Science department, Engineering Science and Mechanics department, the Chemistry and the Physics departments and the Materials Research Laboratory at Penn State.

Resumes, or inquiries should be directed to the following address by no later than September 15, 1985, or until suitable candidates are identified.

**Advisory Committee
Department of Electrical Engineering
Box H, 129 Electrical Engineering East
University Park, PA 16802**

An Equal Opportunity/Affirmative Action Employer.

(NW1909)A

GCRI Physiology & Chemistry Division

3 Year Appointment

Experienced Scientist (HSO/SSO) required to join a team studying the mechanisms through which light regulates plant development, with particular reference to the synthesis and assembly of thylakoid proteins in fully green plants and the regulation of floral induction. Applicants should be experienced in the use of molecular biological techniques.

Qualifications: First or upper second class honours degree with at least two years' post-qualifying relevant research experience for H.S.O. (salary £7,788-£10,541), four years for S.S.O. (salary £9,772-£12,653).

Apply ref. 428/MRB, Glasshouse Crops Research Institute, Worthing Road, Littlehampton, W. Sussex BN17 6LP by 26 July

(7103)A

RESEARCH POSITION

Project Associate/Assistant Scientist position open for candidates with a PhD in immunology. Major areas of research include heart & limb transplantation. Expertise in the biology of endothelium, dendritic cells and lymphocytes preferable. Salary commensurate with experience. Apply to:

Venkat Rao M.D., Department of Surgery, Clinical Science Center, 600 Highland Ave., Madison, WI 53792.

The University of Wisconsin is an affirmative action/equal opportunity employer. (NW36)A

ASSISTANT OR ASSOCIATE PROFESSOR IN PHYSIOLOGY Medical School—full-time faculty

to participate in the human physiology course for freshman medical students and advanced courses for graduate students.

An active research program, preferably in renal, endocrine, neuro- or membrane physiology is highly desired. Rank and salary negotiable depending on qualifications and experience. Starting date: 1 January, 1986.

Send curriculum vitae and three letters of recommendation as soon as possible to:

Dr P. G. Iatridis
Assistant Dean and Director
Northwest Center for Medical Education
3400 Broadway
Gary, Ind 46408

Indiana University is an Equal Opportunity/Affirmative Action Employer (NW37)A

In the course of the expansion of our research effort in the animal health field we are establishing a new R+D unit in

ANIMAL HEALTH BIOTECHNOLOGY

The new unit will be responsible for the preclinical and clinical development of biotechnological derived materials, and will concentrate its efforts in the areas of **NONSPECIFIC IMMUNITY AND GROWTH/PRODUCTIVITY**.

Our organization is committed to establishing and maintaining a highly competitive R+D program in these areas with the ultimate goal of providing novel practical solutions to problems in Animal Health.

As members of our new team we are now seeking several highly qualified Ph.D. or DVM level scientists experienced within the following areas:

- **VIROLOGY**
(Cultivation and biology of viruses affecting livestock)
- **MICROBIOLOGY/PARASITOLOGY**
(Cultivation, biology and immunology of intracellular pathogens affecting livestock)
- **IMMUNOBIOLOGY**
(Immunological methodology, host-pathogen interactions, non-specific immunity)
- **GROWTH PHYSIOLOGY/ENDOCRINOLOGY**
(Hormonal and nutritional factors affecting growth, *in vitro* and *in vivo* test methodology)
- **PHARMACOLOGY**
(Pharmacology of proteins and peptides, immuno-pharmacology, analytical methodology)
- **FORMULATION AND DELIVERY**
(Experimental formulations of proteins, slow release devices, liposomes, adjuvants)
- **PROTEIN BIOCHEMISTRY**
(Isolation and characterization of proteins, recovery process development and scale up, analytical methodology)

Candidates with appropriate qualifications and experience will be considered for group-leader positions.

The new unit will be housed in a separate laboratory building on our Research Station in Switzerland and will profit from the extensive infrastructure and the animal facilities available. The group will engage in collaborations with various internal R+D units, with academic institutions and with leading biotechnology companies worldwide.

Prospective members of the team should bring a solid scientific basis, the capability to function creatively in a highly interactive environment and the willingness to contribute to the solution of practical problems in animal health care.

Applications will be kept confidential and should include curriculum vitae, a list of publications and at least two references.

Please direct your application to Mr. H. Gerber, CIBA-GEIGY LTD., Personnel Department, CH-4002 Basel, Switzerland.

(W2016)A

CIBA-GEIGY

POSTDOCTORAL POSITIONS

Available to study (1) control of lipid metabolism in mammalian hearts, (2) cardiac electrophysiology and pharmacology and (3) cardiac arrhythmias. Background in pharmacology or biochemistry essential.

Send curriculum vitae to Dr R. Man, Department of Pharmacology or Dr P. Choy, Department of Biochemistry, Faculty of Medicine, University of Manitoba, Winnipeg, Manitoba, Canada R3E 0W3.

(NW18)A

POSTDOCTORAL POSITIONS HOWARD HUGHES MEDICAL INSTITUTE/ PHILADELPHIA

Fully funded postdoctoral positions in molecular biology and molecular genetics are now available at the Howard Hughes Medical Institute of the University of Pennsylvania. Active research projects include: transcriptional and translational control of eukaryotic gene expression, mechanisms of cellular transformation and gene amplification, molecular mechanisms of chromatin inactivation and the application of molecular genetics to studies of inherited disease.

For information please contact:

Dr. Steve Liehaber, Coordinator
Howard Hughes Medical Institute
Department of Human Genetics
Medical Labs Building/G3
University of Pennsylvania School of Medicine
Philadelphia, PA 19104

(NW1869)A

SANDOZ LTD.

a leading Swiss-based international company specializing in pharmaceuticals, agrochemicals and dyestuffs, is offering a position for a

**POSTDOCTORAL
RESEARCH SCIENTIST**

for a period of up to two years at its Pharmaceutical Department in Basle, Switzerland, to a person with a recent Ph.D. (or equivalent level).

The position is available as from September 1, 1985 onwards.

This challenging post will involve the development of radioreceptor assays from mammalian cell-lines and its applications to the study of the pharmacokinetics of dopaminergic drugs.

Salary will be competitive. Candidates with a strong background in receptor research are preferred.

Applications with curriculum vitae should be sent to:

SANDOZ

SANDOZ LTD.
Personnel Department, Ref. 8556
attention Mr. R. Zbinden
P.B. CH-4002 Basle
Switzerland

(W2017)A

**DIRECTOR OF MANUFACTURING
FERMENTATION OPERATIONS
BIOTECHNOLOGY**

Israeli Subsidiary of Public U.S. Company

We have been retained by one of the world's leading biotechnology companies to conduct a search for a seasoned Director of Manufacturing from the pharmaceutical or biotechnology industries. Employing genetic engineering and related technologies, our Client is poised to commercialize up to 5 products in the areas of human health care, animal health care and plant agriculture, and has entered into licensing agreements with 3 well-known multi-national pharmaceutical companies. The individual selected for this outstanding growth opportunity must have a strong background in fermentation and protein process manufacturing and will likely be the number 1 person in charge of production at a state-of-the-art facility. The successful candidate will immediately inherit a pilot manufacturing operation of 18-20 supervisory and production personnel and be the key individual leading a design team responsible for scaling up to full manufacturing operations. Quality control and control of the bottom line are both of paramount importance to our Client.

The successful candidate will report directly to the President of the Company and work closely with R&D. A bachelor's degree (master's preferred) in Chemistry, Chemical Engineering or Biology would be a distinct plus. The company offer includes an excellent salary and incentives package.

Please forward your resumé or curriculum vitae, with salary history to:

Mr. Lawrence D. Poster, Vice-President, The Neil
Michael Group, Inc., Suite 631-C, 305 Madison Avenue,
New York, NY, USA 10165.

Executive Search Consultants in Health Care and Science

(NW32)A

**LEONHARD PROFESSORSHIP
IN SOLID STATE DEVICE ENGINEERING**

Applications and nominations are invited for a recently endowed professorship in Solid State Device Engineering within the Department of Electrical Engineering at The Pennsylvania State University. Applicants should have an earned doctorate in electrical engineering, engineering physics, or a related field, and have demonstrated the ability to conduct research and teaching in the field of Solid State Device and Microelectronics. Strong leadership qualities are also emphasized.

The successful applicant is expected to join the existing effort in solid state device research and help expand and develop it into one of prominence in both research and instruction. The College of Engineering has made substantial commitment to support this program including updating of the laboratory facilities and new faculty hiring.

Currently, research in the solid state device and related materials area includes research in silicon device processing, such as plasma and reactive ion etching, thermal nitridation of silicon and various oxide research including ultra-thin oxides, and defect control. In addition, research in III-V device area includes material defects, electrical noise and performance improvement of micron and sub-micron structures.

Combined research in the solid state device area typically amounts to about one million dollars annually. Collaborative research opportunities exist with the Materials Science department, Engineering Science and Mechanics department, the Chemistry and the Physics departments and the Materials Research Laboratory at Penn State.

Résumés, nominations, or inquiries should be directed to the following address by no later than September 15, 1985, or until a suitable candidate is selected.

Chairperson, Leonhard Professor Search Committee
Department of Electrical Engineering
Box J, 129 Electrical Engineering East
The Pennsylvania State University
University Park, PA 16802

An Equal Opportunity/Affirmative Action Employer.

(NW1910)A

NEUROSCIENTIST

With Ph.D. and experience in single-unit recording and/or micro-iontophoresis required to collaborate in ongoing electrophysiological and neurochemical studies of respiration-related brainstem neurones. Position is available immediately.

Send curriculum vitae and names of three references to:

Dr J. L. Henry
Department of Physiology
McGill University
3655 Drummond Street
Montreal
Quebec, Canada H3G 1Y6

Position will be filled when a suitable candidate is found. (NW33)A

POSITION AVAILABLE**Assistant/Associate Professor in Avian Virology**

A vacancy exists for a full-time, tenure track position in the Department of Veterinary Microbiology and Parasitology, Texas A&M University. The incumbent will be responsible for developing an advanced research and teaching program in the molecular biology/biochemistry of avian pathogens. Teaching responsibilities will include instruction and training of graduate, undergraduate and professional students.

A DVM and PhD or PhD degree with research experience in molecular biology/biochemistry of avian pathogens is required. Evidence of a significant publication record and ability to obtain extramural support is also necessary.

Applications will be received until August 15 or until the position is filled.

Applications should be submitted together with a list of publications and names of three references to:

Dr. I. R. Tizard
Department of Veterinary Microbiology
& Parasitology
College of Veterinary Medicine
Texas A&M University
College Station, TX 77843

Texas A&M University is an equal opportunity employer.

(NW34)A

MOLECULAR BIOLOGIST

New, modern research unit emphasizing protein chemistry is seeking an investigator interested in the structure and regulation of eukaryotic genes using recombinant DNA technology. Ph.D. or equivalent and relevant postdoctoral experience required. Available Fall 1985, this position is of indefinite duration dependent upon performance. Salary and rank will be commensurate with experience. If interested, send a current CV and a statement of research interests and goals to:

Sally C. Gay
Personnel Director
Shriners Hospital for
Crippled Children
3101 SW Sam Jackson
Park Road
Portland OR 97201

Equal Opportunity Employer.
 (NW38)A

**Postdoctoral Fellow/
Research Associate**

positions immediately available. Project involves biochemical and molecular studies of cell cycle specific expression of glycolytic enzymes in mammalian cells.

Experience with molecular biological and/or protein chemistry techniques required.

Send curriculum vitae, names and telephone numbers of three references and have one letter of recommendation sent to:

Dr H. Mohrenweiser
4708 Medical Science 2
Box 15
The University of Michigan
Ann Arbor, MI 48109

A Non Discriminatory, Affirmative
 Action Employer (NW24)A

**POSTDOCTORAL
POSITION**

available 10/85 to investigate the effects of chromatin structure on DNA repair in cultured animal cells.

Reply to: Box HOS W218, The Rockefeller University, 1230 York Avenue, New York, NY 10021
 (NW20)A



Public Service Commission
 of Canada

Commission de la Fonction
 publique du Canada

**Research Scientist,
Herbicides**

Agriculture Canada
Canadian Forestry Service
Northern Forest Research Centre
Edmonton, Alberta

We require an experienced scientist to conduct research programs related to the effective and environmentally acceptable use of herbicides for vegetation management. You will provide scientific direction on the use of herbicides for vegetation management in forest ecosystems, and interpret research results to enhance understanding of herbicide residue chemistry, persistence, movement and degradation in selected forest soils under controlled and field conditions. It will be your responsibility to generate scientific knowledge and environmental data as needed.

You require graduation with a doctoral degree from a recognized university, or a lesser degree with evidence of research experience and productivity equivalent to that of a doctoral degree in plant science with specialization in herbicide chemistry and biology. It is essential that you possess experience in herbicide residue chemistry (including the use of gas chromatographs, mass spectrometers and other analytical procedures), as well as in biology and related environmental impact issues. Knowledge of English is essential.

We offer a salary ranging from \$28,176 to \$52,409 commensurate with your qualifications and experience.

Forward your application form and/or résumé, quoting reference number S-85-31-5467-47JG-(G27), to:
Joan Girling (613) 996-8054
Public Service Commission of Canada
Ottawa, Ontario, Canada K1A 0M7

Closing date: 9 August 1985

(NW39)A

The Public Service of Canada is
 an equal opportunity employer

Canada

The University of Texas at Austin

Department of Zoology

PROFESSOR AND CHAIRMAN

The Department of Zoology of the University of Texas at Austin invites applications or nominations for a departmental Chairmanship to be occupied in September of 1986. The Department of Zoology is multi-disciplinary in scope and includes research interests ranging from molecular biology to evolutionary ecology. Specific research interest of the applicant is not of great concern, but an appreciation for the breadth and integration of such diverse biological disciplines, and a strong personal research program is deemed important. Some administrative experience is preferred. Applicants should send a curriculum vitae, a letter elaborating upon research and administrative goals, and the names and addresses of four references to:

Dr F. H. Bronson
Department of Zoology
University of Texas
Austin, Texas 78712

The deadline for applications is November 1, 1985.
 The University of Texas at Austin is an Equal Opportunity/ Affirmative
 Action Employer. (NW35)A

**Assistant or Associate Professorship in
Cardiovascular Pharmacology**

The Stritch School of Medicine, Loyola University of Chicago, is seeking to fill a tenure track position with a cardiovascular pharmacologist. The successful candidate will have a Ph.D. and/or degree, post-doctoral experience, and a capability to develop a productive, independent basic or clinical cardiovascular pharmacology research program.

The Stritch School of Medicine is located in a near-West suburb of Chicago, and has established programs in cardiovascular physiology, cardiac electrophysiology, hematopharmacology, neuropharmacology, and cardiology.

Candidates should send their CV, three letters of recommendation, representative reprints, and a synopsis of their current and future research interests to:

Dr. S. A. Lorens
Department of Pharmacology
Loyola University Medical Center
2160 South First Avenue
Maywood, Illinois 60153
Tel: (312) 531-3261

The deadline for applications is October 1, 1985.
 Loyola University is an Equal Opportunity Employer (NW25)A

THE UNIVERSITY OF ALBERTA
invites applications for the
CANADIAN PACIFIC — ALBERTA
AGRICULTURE CHAIR IN PLANT
BIOTECHNOLOGY

The University of Alberta is seeking an established scientist to occupy the Canadian Pacific — Alberta Agriculture Chair in Plant Biotechnology. The occupant of the chair will be expected to carry out research related to the improvement of agriculture in Western Canada, to provide leadership within the area of plant biotechnology in the Faculties of Agriculture and Forestry, and Science, and to communicate actively with the agricultural sectors of industry and government. Candidates presently located outside the scientific university community will be given serious consideration, although scientific credentials will be of paramount importance.

The successful candidate will be a member of the Department of Plant Science, in the Faculty of Agriculture and Forestry. The Faculty of Agriculture and Forestry has ninety academic members housed in a modern, well-equipped research complex. In addition, within the Faculty of Science, members of the Departments of Botany, Genetics, and Microbiology are currently involved in basic and applied plant research. The University of Alberta (approximately 25,000 students) is located in the capital city of Alberta. Please send curriculum vitae, names and addresses of at least three referees, and an outline of research interests, including reprints of relevant publications, to:

Dr. Mary Spencer
Department of Plant Science
Faculty of Agriculture and Forestry
University of Alberta
Edmonton, Alberta T6G 2P5
Canada

Closing date for receipt of applications is August 31, 1985. Salary will be by negotiation. Appointment will commence on January 1, 1986, or as soon as possible thereafter.

The University of Alberta is an equal opportunity employer but priority will be given to Canadian citizens and permanent residents of Canada. (NW19)A

UNIVERSITY OF DUNDEE
DEPARTMENT OF BIOCHEMISTRY
RESEARCH ASSISTANT
MOLECULAR BIOLOGY

A graduate in Biochemistry or Chemistry with a good honours degree is sought for a research assistantship in the laboratory of Dr David Lilley. The person appointed will join a very active research group working on DNA structure, DNA supercoiling and their relevance to gene expression.

The work will involve chemical synthesis of oligonucleotides, DNA cloning and sequencing, and the study of DNA structure by enzyme and chemical probing and electrophoresis. In addition, immunological and physical methods will be employed.

The laboratory is well funded and this position, which is available immediately, is supported by a grant from the MRC for 3 years. Starting salary £6,600 (under review). Opportunities exist to register for a higher degree.

Informal enquiries may be made to Dr Lilley, (telephone 0382 23181 extension 4243). There is no formal closing date but applications, containing full career details and the names of two referees should be sent as soon as possible to the Personnel Officer, The University, Dundee, DD1 4HN. Please quote reference EST/55/85J. (7084)A

UNIVERSITY OF WALES
COLLEGE OF MEDICINE
DEPARTMENT OF MEDICINE
POST-DOCTORAL
RESEARCH OFFICER

required in the above Department as soon as possible to work in the general field of neuroendocrinology/neurobiology. The areas include the application of radioimmunoassay and HPLC and neurone culture to the study of hypothalamic and other brain neuropeptides. Work also includes the study of the applications of neurotransmitters and neuro-modulators to the control of endocrine function in general and neuroendocrine function in particular. Other methodology involves the measurement of catecholamines by HPLC with electrochemical detection.

Salary on the scale for University Research and Analogous Staff Grade 1A (£7,520-£12,150 per annum, under review); starting point dependent upon qualifications and experience.

Applications, in the form of a curriculum vitae with the names and addresses of two referees (quoting Ref. No. M6/125) should be submitted to the Registrar and Secretary, University of Wales College of Medicine, Heath Park, Cardiff CF4 4XN by July 31, 1985.

Further information available from Dr Maurine Scanlon (Tel No. 0222/755944 ext. 2182). (7086)A

IMPERIAL COLLEGE OF
SCIENCE AND
TECHNOLOGY
DEPARTMENT OF GEOLOGY
RESEARCH ASSISTANT

Applications are invited from persons who wish to join a small research team currently using scanning electron microscopy (JEOL Superprobe) with automated Digiscan image analyser and X-ray energy dispersive spectroscopy, to characterize metal rich particles according to their size, shape and composition. (Microcomputers will be used to improve the resolution of the particle characterization schemes).

In the first instance the object of this research will be to establish a suitable SEM technique for the identification and quantification of lead and other metals in household dust. Several other characterization schemes are being developed, however, and these will be applied to a variety of environmental media.

Applicants (graduates, or equivalent), in a relevant discipline, preferably with a Ph.D., should have experience in operating similar equipment.

This 2 year appointment will be within the RA1A grade £7,520-£12,150 p.a., plus £1,233 London Allowance at a point commensurate with qualifications and experience.

Applicants should send a full C.V. as soon as possible to Dr E. Culbard, Applied Geochemistry Research Group, Department of Geology, Imperial College, Prince Consort Road, London SW7 2BP. Closing date 27 August 1985. Tel. 01-589 5111 ext. 5543. (7110)A

THE UNIVERSITY OF NEW
SOUTH WALES
Sydney, Australia
SCHOOL OF GEOGRAPHY
LECTURER (REF. 1055)

Primary responsibility will be to teach geographic information systems subjects and develop their application in environmental studies in the applied geography programs. Applicants with interests in land evaluation or resource inventory are preferred. A supportive interest in applied remote sensing is desirable.

The position is available from October 1985 and appointment will be on the basis of a contract with provision for conversion to tenure.

Further information from Professor B. J. Garner, Head of School (02) 697-4390, or c/o University of New South Wales, PO Box 1, Kensington, New South Wales, Australia 2033.

Applications close 8 August 1985.

Salary: \$A26,236 range \$A34,467. Commencing salary according to qualifications and experience.

For further information about conditions of appointment and method of application write to the Secretary-General, Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF.

Equality of employment opportunity is University policy. (W2028)A

THE UNIVERSITY OF
WESTERN AUSTRALIA
Perth

LECTURER (TENURED)
(Soil Chemistry)

SCHOOL OF AGRICULTURE (SOIL
SCIENCE AND PLANT NUTRITION)

Applications are invited for appointment as a Lecturer in Soil Chemistry. The appointee would be required to teach in Soil Science units offered in both the Faculties of Agriculture and Science. An ability to teach a wide range of applications of soil chemistry to agriculture is required. The appointee would be expected to develop a research programme in an area of soil chemistry important in agriculture.

Details regarding teaching responsibilities; current research and facilities available can be obtained from Professor A. D. Robson, telephone (09) 380 2505.

Current salary range: Lecturer — \$A26,236-\$A34,467 per annum. Benefits include superannuation, fares to Perth for appointee and dependent family, removal allowance, study leave and long service leave and housing loan scheme.

Applications in duplicate stating full personal particulars, qualifications and experience and the names and addresses of three referees should reach the Staffing Officer, University of Western Australia, Nedlands, Western Australia 6009 by 8 August 1985. Conditions of appointment will be specified in any offer of appointment which may be made as a result of this advertisement. (W2023)A

UNIVERSITY COLLEGE
CARDIFF

DEPARTMENT OF BIOCHEMISTRY
TWO RESEARCH
ASSISTANTS

Applications are invited for the above 3 year posts. The research which is supervised by Dr R J Richards, concerns the biochemical and cellular effects of lung toxins and the prevention of alveolar damage. Duties to commence 1st October, 1985 or as soon as possible thereafter.

A) BIOCHEMIST
Grade 1A/1B

Max starting salary £8,920

B) BIOLOGIST/
BIOCHEMIST Grade 1B

Max starting salary £7,980.

The second post will involve spending some time at CDE, Porton Down, and some experience of EM techniques would be an advantage, but is not essential.

Applications, (2 copies), together with the names and addresses of two referees, should be forwarded to the Establishment Officer, University College, PO Box 78, Cardiff CF1 1XL from whom further particulars will be available. Closing date 16th August, 1985. Ref: 2983 A or B as appropriate. (7063)A

THE UNIVERSITY OF
PAPUA NEW GUINEA

Port Moresby

PROFESSOR OF GEOLOGY
P.071001/85

Applications are invited to fill this post which falls vacant at the end of 1985. Applicants should have proven ability of a high calibre in either Economic Geology/Geochemistry/Igneous Petrology or Palaeontology/Stratigraphy/Sedimentology/Marine environment. The successful applicant must be able to liaise productively with individuals and institutions outside the University, as well as providing leadership in teaching and research within the Department of Geology. Preference will be given to applicants with a genuine interest in extending beyond the initial contract period. The appointee will be expected to become administrative Head of Department as soon as possible.

Salary will be K24,355 per annum plus gratuity

Other conditions: The successful applicant will be normally offered a contract for a three year period ending at the end of a semester. The gratuity is based on 24% of salary earned and is payable in instalments or lump sum and is taxed at a flat rate of 2%. In addition to the salary quoted above, the main benefits include: support for approved research; rent-free accommodation, appointment and repatriation airfares for appointee and dependants; financial assistance towards the cost of transporting personal effects to and from Papua New Guinea; 6 weeks annual recreation leave with home airfares available after each 18 months of continuous service, generous education subsidies for children attending schools in PNG and overseas; a salary continuation scheme to cover extended illness or disability. Applicants wishing to arrange secondment from their home institutions will be welcomed. The terms and conditions are under review.

Applications will be treated as strictly confidential and should include a full curriculum vitae, a recent small photograph and the names and addresses of three referees. In order to expedite the appointment procedure, applicants are advised to ask their referees to send confidential reports directly to the University without waiting to be contacted. Applications should be forwarded to the Deputy Registrar (Staffing), University of Papua New Guinea, PO Box 320, University PO, Papua New Guinea, and close on 6 August 1985. Candidates in UK should also send a copy of their application to the Secretary General, Association of Commonwealth Universities (Apts), 36 Gordon Square, London WC1H 0PF. (W2026)A

Please mention

nature

when replying to
these advertisements

FERMENTATION MICROBIOLOGIST

Drug Research

Pfizer is one of the largest and most successful research-based international pharmaceutical companies. In our modern, well-equipped and recently extended laboratories at Sandwich on the Kent coast about 600 scientists and support staff are engaged in the discovery and development of novel compounds for use in human and veterinary medicine.

Our Animal Health group, currently 90 strong, has a major commitment to fermentation as an approach to the discovery of new agents for improving the health and productivity of farm livestock.

We need a creative scientist to contribute to the discovery of novel biologically active agents and to take responsibility for all aspects of our culture maintenance and modification programme.

For this important new position we are seeking a practical Microbiologist with up to three years postdoctoral experience in Actinomycete biology, microbial physiology/biochemistry or genetic techniques who is capable of interacting effectively with other scientists both inside and outside the organisation.

This appointment offers excellent opportunities for personal and career advancement and a highly competitive starting salary together with good bonus, pension and life assurance schemes, generous assistance in relocating to an attractive semi-rural area of coastal Kent, flexible working hours and an active sports and social club with first-class amenities.

Please write in confidence quoting ref N1 giving full details of career to date to Mr D. O'Callaghan, Pfizer Central Research, Ramsgate Road, Sandwich, Kent CT13 9NJ. (7100)A

Pfizer

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE

(University of London)

ACADEMIC DEPARTMENT OF
HAEMATOLOGY

RESEARCH ASSISTANT

Applications are invited for the post of Research Assistant with the Bone Marrow Transplantation Team. Applicants should have MSc or equivalent, with undergraduate training in biosciences and postgraduate training in human pathology particularly haematology. Work experience in clinical and laboratory haematology desirable.

The successful candidate will be responsible for collection, collation and analysis of data for clinical studies; preparation of reports, abstracts and papers, and for investigation of new developments or problems.

Salary on scale £6,000-£8,920 (under review) + £1,233 London Allowance according to qualifications and experience.

Further details available from the School Office, R.F.H.S.M., Rowland Hill Street, London NW3 2PF (01-794 0500 ext. 4262) to which applications (full curriculum vitae and names and addresses of two referees) should be sent by 26 July 1985. Please quote reference AVH/RA. (7099)A

UNIVERSITY OF GUELPH

DEPARTMENT OF ANIMAL AND POULTRY SCIENCE

The Department of Animal and Poultry Science, University of Guelph, seeks faculty members at the assistant/associate rank for tenure-track positions in molecular genetics or biochemical genetics.

The successful applicants will be expected to develop strong research programs in gene transfer and the expression of genes in developing avian and/or mammalian embryos. Major emphasis will include mediation of gene transfer by retroviral transduction, recombinant DNA techniques, gene cloning, splicing and transfer. The appointees will be expected to be an integral part of a university team approach to genetic engineering of farm animals.

In addition to establishing a vigorous laboratory, the incumbents will also be expected to participate in the ongoing undergraduate and graduate teaching programs of the department.

A Ph.D. degree with demonstrated expertise in molecular genetics or a related field is required. Applicants should submit curriculum vitae, bibliography, recent reprints, transcripts, and three letters of reference to:

**Dr. G. K. Macleod, Acting Chairman
Department of Animal & Poultry Science
University of Guelph
Guelph, Ontario, Canada N1G 2W1**

Closing date for receipt of applications is August 30, 1985 or until the positions are filled.

Citizenship is not a factor for consideration.

Subject to final budgetary approval.

(NW29)A

SANDOZ LTD.
Biopharmaceutics Department

NATURAL SCIENTIST OR PHARMACEUTICAL TECHNOLOGIST

required to work on pharmacokinetic aspects of new drug development.

In conjunction with medical, bioanalytical and drug-formulation specialists, your brief will be to plan studies in clinical and preclinical phases and to evaluate the results using both pharmacokinetic/pharmacodynamic and statistical models. Methodological innovation will be encouraged.

Applicants should have a good grasp of the fundamentals of pharmacokinetics and an understanding of biological problems, plus a knowledge of EDP and statistics. Post-doctoral research or industrial experience would be an advantage. A knowledge of German is also desirable.

Candidates with appropriate qualifications are invited to submit their written applications to:

SANDOZ

SANDOZ LTD.
Personnel Department, Ref. 8554
CH-4002 Basle
Switzerland

(W2018)A

DEPUTY DIRECTOR, GENERAL SCIENCES Salary Open

Duties: Reporting to the Laboratory Director, the Deputy Director of General Sciences provides overall direction for Laboratory divisions in high energy physics, nuclear physics, accelerator physics, and fusion research. Major responsibilities include management oversight of these divisions, coordination of inter-divisional programs and activities, establishment and implementation of goals consistent with the Laboratory's mission, interaction with policy and advisory committees to ensure the highest quality of scientific achievement, and management liaison with the University of California, the U.S. Department of Energy, and other external agencies.

Requirements: A distinguished record of scientific achievement and proven leadership in managing interdisciplinary programs in fields such as high energy physics, nuclear physics, accelerator physics, or fusion research. Proven success in interacting with academic institutions, scientific laboratories, private industry, and the federal government (especially the U.S. Department of Energy). Must have a Ph.D. in the physical sciences or equivalent experience. Requires strong management skills in areas of decision-making, problem-solving, interpersonal communications, staffing, funding, and facilities planning and utilisation. "Position is subject to the financial disclosure requirements of the California Political Reform Act of 1974."

Please submit two copies of resumé including salary history and names and addresses of references to:

Chairman, Search Committee
Lawrence Berkely Laboratory
Employment Office
1 Cyclotron Road
Building 90, Room 1012
Berkeley CA 94720

Refer to Job#B/3425. Resumés must be received in the Employment Office by September 1, 1985, 5.00 p.m. *An Equal Opportunity Employer M/F/H.* (NW14)A

MASSEY UNIVERSITY
Palmerston North, New Zealand
DEPARTMENT OF BOTANY
& ZOOLOGY

LECTURESIPS

Applications are invited for two lectureships in the above Department. Consideration will be given to suitably qualified applicants, but preference may be given to those with research experience and/or special interests in:

BOTANY

- (i) Plant cell biology
- (ii) Population biology
- (iii) Plant genetics
- and/or Plant biosystematics

ZOOLOGY

- (i) Population biology
- (ii) Invertebrate zoology (except Insects)
- (iii) Aquatic zoology
- (iv) Development biology

The appointees will be expected to teach internal and extramural students at the undergraduate level, contribute to postgraduate teaching, and establish and develop research in their own field of interest.

Salary on the Lecturer's scale: NZ\$23,622-\$27,928.

Conditions of appointment are available from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF; or from the Registrar of the University, with whom applications close 30 August 1985. (W2025)A

WHO COLLABORATING CENTRE CHELSEA HOSPITAL FOR WOMEN RESEARCH ASSISTANT

Applications are invited for the above post to work on the development of an immunoassay for gossypol, a potential male antifertility agent.

Applicants (post doctoral or equivalent experience) should have experience in the development of immunoassays for low molecular weight analysis. Some knowledge of chemical syntheses would be advantageous.

The post is available immediately for a period of one year with the possibility of extension. Salary in the 1A Range for Research and analogous staff (£7,520-£9,390 plus London Weighting).

Applications (curriculum vitae plus the names of 2 referees) should be sent by 24 July to: WHO Collaborating Centre for Immunoassay, Chelsea Hospital for Women, Dovehouse Street, London SW3 6LT.

Informal enquiries may be made to: Mr S Sufi or Dr A Donaldson (telephone 01-351 9336). (7091)A

**UNIVERSITY OF
LEICESTER**
DEPARTMENT OF PHYSICS
RESEARCH ASSOCIATE

Applications are invited for a SERC-funded research associateship for work on the theory of reflection high energy electron diffraction (RHEED). This is a rapidly developing area of surface science with important applications in semiconductor growth. The successful applicant will be expected to develop the theory and computer programs used for RHEED intensity analysis. This will involve close contact with experimental groups working in industrial and academic research. Candidates should have a Ph.D. or expect to obtain one shortly. Experience of either computational solid state physics or of interpreting experimental data would be an advantage. The post is tenable for up to 3 years from 1st October or as soon as possible thereafter, and the initial salary will be £7,520 or £7,980 per annum (under review) depending on age and experience. Applications, including a CV and the names of two referees, should be sent to Dr P A. Maksym, Department of Physics, University of Leicester, Leicester LE1 7RH, as soon as possible. (7096)A

UNIVERSITY OF GLASGOW
DEPARTMENT OF
PATHOLOGY
WESTERN INFIRMARY

A Ph.D. graduate with 3 years' post-doctoral research experience in protein chemistry is required for a three year Arthritis and Rheumatism Council project grant in the University of Glasgow. The aim of the project is to isolate and characterize a plasma protein which interferes with the complement-mediated solubilisation of antigen-antibody complexes. Salary, on the University Scale 1A. Applications with C.V. and names and addresses of two referees to Professor K. Whales, Department of Pathology, Western Infirmary, Glasgow G11 6NT by 25th July, 1985. (7097)A

**UNIVERSITY OF
CAMBRIDGE**
DEPARTMENT OF
HAEMATOLOGICAL MEDICINE
RESEARCH ASSOCIATE

Applications are invited from post-doctoral or otherwise experienced graduates to participate in a research programme involving the cytogenetic analysis of abnormal clones and detailed determination of breakpoints in structural rearrangements in myeloma and their relation to oncogene activation and paraprotein production.

Salary on an age-related scale, e.g. £7,980 at age 25 to £10,330 at age 30. Applications with c.v. and names of two referees to Professor F. G. J. Hayhoe, Department of Haematological Medicine, Hills Road, Cambridge CB2 2QL before 31 July, 1985. (7104)A

THE UNIVERSITY OF LEEDS

DEPARTMENT OF BIOCHEMISTRY POSTDOCTORAL RESEARCH FELLOW

Applications are invited, as soon as possible, for the above post for the study of haem metabolism in plants and its significance in the synthesis and organisation of photosynthetic pigments. The successful applicant will join a group studying various aspects of tetrapyrrole biochemistry and their application to medical and biological problems.

The post is funded by the SERC and is available for a fixed period of up to three years from a starting date to be agreed. Applicants should hold, or expect to hold shortly, a PhD in a relevant discipline.

Informal enquiries may be made to Dr S B Brown (Tel 0532 431751 ext 7500).

Salary on the IA Range of Research and Analogous Staff (£7,520 — £12,150) (under review) according to age, qualifications and experience.

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT quoting reference no 83/64. Closing date for applications 31 July 1985. (7073)A

ENZYMOLOGIST

Applications are invited from persons with experience in the semi-commercial production of enzymes (pilot plant scale).

Successful applicant would be expected to organise team for new projects. Write giving full details of past experience: Maybridge Chemical Co Ltd Tintagel, N Cornwall PL34 0HW. (7076)A

UNIVERSITY OF OXFORD Research Laboratory for Archaeology

POST-DOCTORAL POSITION IN CHEMISTRY

A post is available, tenable for 3 years from October 1985 for a post-doctoral chemist to develop methods in radiocarbon dating

A 2MV Accelerator Mass Spectrometer is used for routine and research dating of milligram-sized organic samples. Improved methods of sample analysis, purification and ion source target preparation, and better understanding of the effects of burial environments are sought.

Applicants should have some research experience, preferably in organic chemistry. Salary will be on Scale 1A, Points 1-5 (£7,520 — £9,390) depending on age and experience. Applications should be made to Dr R E M Hedges, Director, Radiocarbon Accelerator Unit, Research Laboratory for Archaeology, 6 Keble Road, Oxford OX1 3QJ, and should include details of qualifications and experience and the names of two referees. (7059)A

THE UNIVERSITY OF WESTERN AUSTRALIA Perth

LECTURER (LIMITED TENURE) (Soil Microbiology)

SCHOOL OF AGRICULTURE (SOIL SCIENCE AND PLANT NUTRITION)

Applications are invited for appointment as a Lecturer in Soil Microbiology for a term of three years. The appointee would be required to teach in both Soil Science and Agricultural Microbiology units in the Faculties of Agriculture and Science. An ability to teach a wide range of applications of soil microbiology to agriculture is required. Research interests in any of the areas of rhizobial ecology, mycorrhizal symbioses, or microbial involvement in chemical transformations in soils would be desirable but not essential.

Details regarding teaching responsibilities, current research and facilities available can be obtained from Professor A. D. Robson, telephone (09) 380 2505.

Current salary range: Lecturer — \$A26,236-\$A34,467 per annum. Benefits include superannuation, fares to Perth for appointee and dependent family, and removal allowance.

Applications in duplicate stating full personal particulars, qualifications and experience and the names and addresses of three referees should reach the Staffing Officer, University of Western Australia, Nedlands, Western Australia 6009 by 8 August 1985. Conditions of appointment will be specified in any offer of appointment which may be made as a result of this advertisement. (W2024)A

UNIVERSITY OF ABERDEEN

DEPARTMENT OF PATHOLOGY RESEARCH FELLOW/ ASSISTANT IN TUMOUR IMMUNOLOGY

Support is available for 1 year from 1 October 1985 for a suitable individual to work on the characterization of anti-inflammatory factors generated during tumour growth. The post, which is funded by the Cancer Research Campaign, is suitable for applicants who have or are about to obtain a Ph.D. in biochemistry, immunology or cell biology, or to candidates with a good first degree in either of these subjects. Some experience in protein fractionation/characterization would be an advantage but is not essential.

Salary within range £7,520-£9,860 per annum on the Range 1A Scale for Research and Analogous Staff with appropriate placing (scale under review).

Further particulars and application forms from The Secretary, The University, Aberdeen, with whom applications (2 copies) should be lodged by 2 August 1985. (Ref EA/008). (7094)A

BIOGEN

is an international biotechnology company with laboratories in Switzerland, Belgium and the United States in the development of new pharmaceutical products through genetic engineering.

Vacancies now exist in our Geneva facility for well qualified scientists who are skilled in the areas of:

Cell Biology
Protein Chemistry
Fermentation Technology
Immunology

Post doctoral positions are also available in these areas.

We offer a challenging scientific career with the opportunity to interact with talented colleagues in a stimulating environment.

If this offer appeals to you, then reply to:

Rita Gloor, Personnel Manager
BIOGEN S.A.
route des Acacias 46
1227 Geneva
Switzerland

Please send your resumé and a list of three individuals who may be contacted for references on your behalf. All replies will be evaluated in confidence. (W2015)A



National Research
Council Canada

Conseil national
de recherches Canada

Yeast Geneticists Montréal, Québec

Positions are available, at the post-doctoral level, in a group studying the molecular genetics of the yeast *Saccharomyces cerevisiae*. Qualified candidates with experience or an interest in gene expression and protein secretion are invited to apply.

The group is working within the new Biotechnology Research Institute located close to McGill University in Montréal and has several collaborations with universities, industry and other research institutes. The Institute has several groups working on molecular genetics and is superbly equipped.

Benefits are fully competitive and salary will be commensurate with qualifications and experience.

Please send a detailed curriculum vitae with 3 references to: The Manager, Employment Office, National Research Council of Canada, Ottawa, Ontario, K1A 0R6. In reply please quote RB-84-25. (NW40)A

Canada

THE UNIVERSITY OF CALGARY ASSISTANT PROFESSOR IN MOLECULAR BOTANY

The University of Calgary, Department of Biology, invites applications for a tenure-track position in Molecular Botany at the assistant professor level. While the successful candidate should be able to complement and co-operate with existing research programs in plant and cell biology and plant physiology, the main goal is to develop a strong independent research program in the molecular biology of plants. Current areas of interest in the Department are plant growth and development, and the molecular basis of the response of plants to stress, but the candidate is not restricted to these. Experience with the techniques of recombinant DNA and related technologies is essential.

Teaching duties will include lecturing in an introductory general botany course and in senior courses concerned with the biochemical/molecular aspects of higher plants.

A well equipped research laboratory is available. Other equipment is also available in plant physiological and molecular biological laboratories within the Department.

The appointment will be open as of December 1, 1985, with a starting salary in the range of \$29,741 — \$34,489 (currently under review).

All qualified individuals are encouraged to apply but preference will be given to Canadian citizens and permanent residents.

Applicants are requested to submit a complete curriculum vitae, and to have three letters of academic reference forwarded by September 30, 1985 to: Dr. R.W. Davies, Head, Department of Biology, The University of Calgary, 2500 University Drive N.W., Calgary, Alberta, Canada T2N 1N4 (NW13)A

UNIVERSITY OF BATH SCHOOL OF CHEMISTRY

RESEARCH OFFICER IN MOLECULAR GRAPHICS

This is a 3 year position, as a member of a newly-established interdisciplinary unit within the science area, located in the School of Chemistry. The successful candidate will undertake research in any appropriate area within chemistry and will also assist, on a fully collaborative basis, researchers from other schools, including pharmacy and pharmacology, and biochemistry.

The unit will be equipped with an Evans and Sutherland PS330 micro VAX II. Access to mainframe computers is available. Links to a network of computers are planned.

Applicants should have an appropriate background in molecular graphics or in a relevant area.

Salary in the range £7,520–£12,150 p.a. according to qualifications and experience.

Informal contact may be made with Professor M. M. Campbell, School of Chemistry (0225 61244 ext. 565).

Further particulars and application forms from the Personnel Officer, University of Bath BA2 7AY, quoting Ref. No. 85/108. Closing date: 30-7-85. (7108)A

RESEARCH FELLOW

1 year term appointment,
subject to renewal

Accession and catalog a fossil fish collection from Brazil. Also write factual text for illustrated atlas and catalog, and conduct systematic research on species in the collection.

The ideal candidate will have the following credentials: 1) PHD in the natural sciences area desired; 2) A background in ichthyology or paleoichthyology; 3) Previous publications experience. A knowledge of bony fishes also is desired.

The starting salary is \$17,000 per annum with excellent benefits. To apply, send résumé along with references to: Jerome Williams, Personnel Department.



American Museum of Natural History

Central Park West at 79th Street
New York, NY 10024

An equal opportunity (M/F/H)
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(NW15)A



Athrofa Gogledd Ddwyrain Cymru The North East Wales Institute of higher education

RESEARCH DIVISION, DEESIDE, CLWYD
CLWYD HEALTH AUTHORITY

RESEARCH TECHNICIAN

ANAESTHESIA AND IMMUNOCOMPETENCE
(SCALE 3) (£5,922 — £6,420)

A Research Technician is required, for one year in the first instance, to continue an investigation of the effects of anaesthesia on lymphocyte function. This is a collaborative project between The North East Wales Institute and the Department of Anaesthetics, Maelor General Hospital, Wrexham. Applicants should be qualified and experienced in cell biology, immunology, biochemistry or haematology.

Application forms and further details available from The Registrar, The North East Wales Institute, Connah's Quay, Clwyd. Tel: Deeside (0244) 817531 Extn. 254. Closing date for receipt of applications 26th July 1985. (7083)A

dkfz

Project FLOW CYTOMETRY OF MALIGNANT HUMAN TUMORS

(5 years)

offers postdoctoral position (for 2 years, prolongation possible according to validity) for scientist up to 35 years.

Experience in quantitative and analytical cytology is preferred.

Salary commensurate to experience and according to the rules of German Scientific Service.

Applications including CV, research experience, interests, publications, to be sent to:

Prof. Dr. Klaus Goerttler
Institute of Experimental Pathology
DKFZ

Im Neuenheimer Feld 280
D-6900 Heidelberg 1

(W2021)A

BIOTECHNOLOGISTS!

What's going on in the world's fastest-growing scientific discipline?

To find out, send for a sample copy of **BIO/TECHNOLOGY**, the International Monthly for Biology and Industry, to

Felicity Parker, Macmillan Journals,
Houndmills, Basingstoke, Hants.
RG21 2XS, UK

or

Paul Siman, Bio/Technology,
65 Bleecker St., New York, NY 10012.

**THE MIDDLESEX
HOSPITAL MEDICAL
SCHOOL**

London W1P 7PN

**THE COURTAULD
INSTITUTE OF
BIOCHEMISTRY**

**POST-DOCTORAL
RESEARCH ASSISTANT**

Applications are invited for the position of Post-Doctoral Research Assistant, funded by the CRC for 3 years from 1 October 1985, to study the expression of cellular genes which respond to the infection of human B-lymphocytes with Epstein-Barr Virus. The post will be in a well-equipped medical molecular biology unit within the School. Experience with recombinant DNA techniques would be advantageous, but not essential. Salary within the range £8,753-£10,153. Applications, including CV and names of 2 referees, to Dr P. M. Brickell at the above address. (7111)A

BIOCHEMIST/CHEMIST

G.D. Searle is a major international pharmaceutical company whose Research & Development Division at High Wycombe is involved with the discovery of new chemical and biological entities.

A vacancy has arisen within our PROTEIN ENGINEERING DEPARTMENT for a graduate scientist to join an established team involved in the design and production of modified proteins using recombinant DNA techniques. Experience of oligonucleotide synthesis or the enzymatic manipulation of DNA would be an advantage.

The Company offers competitive salaries, 23 days holiday, contributory pension fund, health insurance scheme, subsidised cafeteria, recreation club and modern offices in a very pleasant location. Generous relocation assistance will be available in appropriate cases.

Please apply to the Personnel Manager, Searle Research & Development, Lane End Road, Sands, High Wycombe, Bucks. (Tel. High Wycombe 21124, Ext. 3372). Please quote reference R30/136.

(7112)A

SEARLE

UNIVERSITY OF ULM

MOLECULAR BIOLOGIST or PROTEIN CHEMIST

Applications are invited for PRE- OR POST-DOCTORAL RESEARCH FELLOWSHIPS funded for three years commencing January 1st, 1986 (Salary according to BAT II a/b). A research group will focus on the expression of oncogenes during regulation and dysregulation of cell growth and cell transformation by the DNA-tumor virus SV40. Applicants should have experience in DNA technology and analysis of gene expression or alternatively in protein chemistry.



Applications including CV and the names of two referees should be sent to Prof. R. Henning, Department of Biochemistry, University of Ulm, P.O. Box 4066, D-7900 Ulm, West Germany.

(W2013)A

DIRECTOR

BERMUDA BIOLOGICAL STATION

Applications are invited for the position of Director of the Bermuda Biological Station for Research Inc.

The Director is the chief executive officer of the Station, with responsibility for all day-to-day operations, planning, and overall enhancement of scientific programs. The Director should have a Ph.D. in marine science and experience in administration and research funding. Responsibilities include financial management, development and public relations, recruiting and evaluation of senior staff, and maintenance of facilities. Salary is negotiable.

The Station is an independent U.S. not-for-profit marine laboratory with facilities for resident and visiting scientists and students. There are research and teaching laboratories, a small research fleet, workshops, an excellent library, a staff of 35 and accommodations for up to 140.

Send applications to W. R. Wright, BBS President, Box 54, Woods Hole, MA 02543, U.S.A. (NW30)A

ANGLIAN BIOTECHNOLOGY LTD

We are a progressive research and development company specializing in recombinant DNA technology for research, and diagnostic purposes.

We are based in Colchester, a town with fast and frequent connections to London, and have modern air conditioned facilities. We have large scale fermentation and production units as well as an active R & D programme.

We require:

- Microbiologist/ Fermentation specialist** for responsibility in cell culture and down stream processing.
- Postdoctorate and/or graduate Molecular Biologists.**

Please apply in writing with full CV to:

**ANGLIAN BIOTECHNOLOGY LTD,
HAWKINS ROAD,
COLCHESTER,
ESSEX CO2 8JX,
ENGLAND**

(7092)A

UNIVERSITY OF MANITOBA Department of Human Genetics

MOLECULAR GENETICIST

The Department of Human Genetics, Faculty of Medicine, University of Manitoba, invited applications for a full-time Assistant Professor to commence as soon as possible and certainly by January 1, 1986. Applicants should have demonstrated research experience in Molecular Genetics, particularly in the areas of genome organization, control of gene expression or gene mapping. A Ph.D., post-doctoral training and potential for an independent career as an experimental scientist are prerequisites.

The university encourages both women and men to apply for positions. In accordance with Canadian immigration requirements, this advertisement is directed to Canadian citizens and permanent residents.

A concise description of projected research goals and interests, the names of at least three referees, and a curriculum vitae should be sent to Dr John L. Hamerton, Department of Human Genetics, Faculty of Medicine, University of Manitoba, 250-770 Bannatyne Avenue, Winnipeg, Manitoba, Canada R3E 0W3. (NW22)A

THE POLYTECHNIC WOLVERHAMPTON

LECTURER II IN CHEMISTRY AND COMPUTING (temporary)

The post involves lecturing in organic and/or physical chemistry. The successful applicant will be expected to participate in team research in involving computer-controlled automated solid phase peptide synthesis

RESEARCHER A IN ORGANIC CHEMISTRY (temporary)

This post involves in ultra-high solid (gel) phase peptide synthesis. The successful applicant will be expected to register for a CNA A MPhil or PhD degree

RESEARCH ASSISTANTS (2 posts — 3 years) RESEARCHER A

Metals in gastrointestinal physiology and pharmacology and metabolism of intestinal peroxisomes. Previous research experience an advantage for one post in inorganic pharmacology. For the other post, experience of subcellular fractionation and NMR preferred but not essential. Registration for MPhil/PhD (CNA A) is expected

POST-DOCTORAL RESEARCH FELLOW (1 post - 2 years) RESEARCHER B

Required for study on metal-ion interactions in intestinal absorption. Experience in gastrointestinal physiology/pharmacology essential. Familiarity with AAS, XRF, NMR, TEM, and SEM techniques an advantage. Interdisciplinary approach essential.

Informal enquiries to Dr N J Birch. Salary Scales: Lecturer II — £7,548 — £12,099; Researcher A — £5,910 — £6,657; Post-doctoral Research Fellow — £7,548 — £10,251.

Further details and application forms from The Staffing Office, The Polytechnic, Wolverhampton WV1 1SB or telephone Wolverhampton (0902) 710654 (ansaphone) (7080)A

UNIVERSITY COLLEGE
CARDIFFDEPARTMENT OF METALLURGY
AND MATERIALS SCIENCE
LECTURER

Applications are invited for the above post. Preference will be given for someone prepared to research into non-metallic materials. The appointment will be made at the lower end of Lecturer's scale £7,520 — £14,925 per annum. Duties to commence as soon as possible.

Applications (3 copies), together with the names and addresses of two referees, should be forwarded to the Vice-Principal (Administration) & Registrar, University College, PO Box 78, Cardiff CF1 1XL from whom further particulars are available. Closing date 9th August, 1985. Ref: 2985. (7060)A

UNIVERSITY OF DURHAM

Plant Molecular Biology Group, to work on the molecular basis for control of seed protein gene expression in pea. This work will form part of a continuing programme on proteins in legume seeds and their encoding genes, and will involve gene transfer techniques in plants. Experience in working with plant nucleic acids of *Agrobacterium* gene transfer systems would be an advantage, but is not essential.

The post funded by the SERC and tenable for three years from October 1st 1985, or as soon as possible thereafter.

Initial salary £7,520 — £8,920 (under review) on Range 1A with superannuation.

Applications (3 copies) naming three referees to be returned by 26 July 1985 to the Registrar, Science Laboratories, South Road, Durham, DH1 3LE, from whom further particulars may be obtained.

(7067)A

INSTITUTE OF
OBSTETRICS AND
GYNAECOLOGY

(University of London)

POSTDOCTORAL
RESEARCH

Applications are invited for a three-year postdoctoral research post, funded by the Parkinson's Disease Society, to study the mechanism of action of the neurotoxin, MPTP. The work will be both chemical and anatomical and some background in either could be appropriate. Starting salary £10,143 p.a. Please send curriculum vitae, together with the names of two referees, to Professor Merton Sandler, Queen Charlotte's Maternity Hospital, Goldhawk Road, London W6 0XG. (7109)A

JOHN INNES INSTITUTE

Temporary Appointment
SCIENTIFIC OFFICER

required in the Department of Virus Research to assist with research involving protoplasts, cell culture and transformation, in a group investigating the construction of gene vectors involving plant DNA virus sequences. The research group is directed by Dr J W Davies from whom further particulars may be obtained.

Candidates should have a degree of HNC and preferably experience in molecular biology and/or plant tissue culture research

This post is available from August, 1985. Salary scale £6,190-£8,561. Starting salary depending on experience.

Applications giving full particulars and experience, the names of two referees, quoting reference VIR/201, should be sent to the Secretary, John Innes Institute, Colney Lane, Norwich NR4 7UH

(7087)A

TWO POSTDOCTORAL POSITIONS

are available immediately for a Ph.D. biochemist and a molecular biologist to participate in studies of gene regulation by steroid receptors. Projects include the purification and characterization of human estrogen and progesterone receptors, cloning of the corresponding genes and preparation of monoclonal antibodies. Experience with steroid receptors, protein purification and analysis, monoclonal antibody production and/or recombinant DNA techniques is desired. Salary is negotiable

Send curriculum vitae and three letters of references to: Dr Geoffrey L. Greene, The University of Chicago, Ben May Laboratory for Cancer Research, 5841 S. Maryland, Chicago, Illinois 60637. (NW1903)A

UNIVERSITY OF
BIRMINGHAMFACULTY OF MEDICINE
AND DENTISTRY

DEPARTMENT OF PHYSIOLOGY

Applications invited for a three year post graduate appointment, funded by the British Heart Foundation to work on brain Angiotensin and the central nervous regulation of blood pressure. The work will involve a range of techniques in neurophysiology, immunohistochemistry, and neurochemistry. Experience in one of these areas an advantage.

Salary in range £6,600-£8,920 with superannuation

Further details from Professor J. H. Coote, Department of Physiology, The Medical School, Birmingham B15 2TJ.

Applications (3 copies) with C.V., lists of publications and names of two referees to Assistant Registrar, Medical School, Birmingham B15 2TJ by 5 August 1985. Quote ref RA/Phy/JHC. (7093)A

THE UNIVERSITY
OF SUSSEXLABORATORY OF
EXPERIMENTAL
PSYCHOLOGY

Algorithms for Speech Analysis

Applications are invited for two 3-year research posts, one at post-doctoral and one at postgraduate level, to join a group funded by SERC/Alvey working on algorithms for the analysis and recognition of speech. Applicants should have experience in at least one of the following areas: digital signal or image processing, programming in high-level languages, phonetics, or speech perception.

The posts start on 1 October 1985 and will be on the scale £6,600 to £8,920 per annum for postgraduate, and £7,520 to £12,150 per annum for post-doctoral, with starting salary not above £11,675.

Applicants should send a CV to Dr C. J. Darwin, Experimental Psychology, University of Sussex, Brighton BN1 9QG. Tel: 0273 606755 ext. 54. (7090)A

UNIVERSITY OF
ST ANDREWSDEPARTMENT OF
BIOCHEMISTRY AND
MICROBIOLOGYApplications are invited for a post o
RESEARCH ASSISTANT IB

from graduates in Biochemistry Chemistry or Microbiology. The position is funded by the SERC for 2 years and is for an investigation into the biochemistry of the sulphur and ferrous iron oxidizing systems of *Thiobacillus ferrooxidans*. The person appointed may register for a higher degree

Starting salary within range £6,600-£7,055 on IB scale, plus USS

Further details can be obtained from Dr W J Ingledew, Department of Biochemistry and Microbiology (Telephone 0334 76161 ext. 270). Applications (2 copies preferably in typescript) with the names of three referees should be sent to the Establishments Officer, College Gate, St Andrews, Fife KY16 9AJ to arrive not later than August 5 1985. (7088)A

THE SCHOOL OF
PHARMACY

University of London

MRC

NEUROPHARMACOLOGY
RESEARCH GROUPApplications are invited for a
**POST-DOCTORAL
RESEARCH ASSISTANT**

to work with Dr Sandra File on a multi-disciplinary project on the neural basis of anxiety. Experience in HPLC and ligand binding techniques preferred.

The post is available from August for 3 years and is financed on the Wellcome Trust. Starting salary £8,753 pa inclusive of London Allowance

Applications, quoting Ref: SEF including 2 copies of CV and names of 2 referees, should be sent as soon as possible to: Personnel Officer The School of Pharmacy, 29/39 Brunswick Square, London, WC1N 1AX.

Further details can be obtained from Dr S File (tel: 01-837 7651 ex 4). (7078)A

nature
classified

LONDON OFFICE

4 Little Essex Street
London WC2R 3LF
Tel 01 240 1101
Telex 262024

TORONTO OFFICE

Peter Drake & Associates
17 Pine Crescent
Toronto, Ontario M4E 1L1
Tel (416) 690-2423

NEW YORK OFFICE

65 Bleecker Street
New York, NY 10012
Tel: (212) 477-9600

POST-DOCTORAL RESEARCH ASSISTANT IN ASTRONOMY

DEPARTMENT OF ASTROPHYSICS

OXFORD UNIVERSITY

It is expected that an SERC-supported post will be available from October 1985 for a Post-Doctoral Research Assistant in the Astrophysics Department to work on the observation and interpretation of cataclysmic variables and related objects. The post is tenable for up to 3 years at a starting salary of up to £8,450 (age point 26), which is currently under review.

Applicants should send a resumé and themselves request 2 referees to write to: Dr. P.A. Charles, Department of Astrophysics, University of Oxford, South Parks Road, Oxford OX1 3RQ, UK.

Deadline for receiving applications is August 30, 1985. (7081)A

UNIVERSITY OF EAST ANGLIA

Norwich

POST-DOCTORAL POST IN PLANT SCIENCE

A 3 year position funded by the AFRC is available to work on biochemical aspects of the effects of anoxia on plants. Candidates should have a PhD in biochemistry/physiology. The project involves collaboration between the University of East Anglia and the John Innes Institute.

Enquiries and applications, including full CV and the names of three referees should be addressed to Professor D D Davies, School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ. Applications should be received within 2 weeks of the publication of this notice. (7068)A

UNIVERSITY OF LEICESTER

DEPARTMENTS OF BIOCHEMISTRY AND CHEMISTRY

2 POSTDOCTORAL RESEARCH ASSOCIATES MOLECULAR BIOLOGIST AND BIOLOGICAL CHEMIST TO INVESTIGATE MECHANISMS OF RNA PROCESSING

Applications are invited for these two SERC-funded posts to carry out research on eukaryotic RNA processing mechanisms using nucleotide analogues, in collaboration with Drs I. C. Eperon (Biochemistry) and B. V. L. Potter (Chemistry).

Candidates should possess a Ph.D. and experience or interest in molecular biology or nucleic acid chemistry (training in molecular biological techniques will be provided).

The posts will be tenable for three years, and salary will be within the range £7,520-£12,150 p.a., depending on qualifications and experience. Applications (including a c.v. and names of two referees) should be sent to either of the above at University of Leicester, Leicester LE1 7RH, U.K., to arrive not later than 16 August (7105)A.

NOTTINGHAM UNIVERSITY

DEPARTMENT OF BIOCHEMISTRY POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for a post-doctoral position to carry out protein engineering of an E.coli vitamin B12-requiring enzyme. The work will involve the use of genetic and biochemical methods of gene manipulation (including site-specific mutagenesis) to define amino acid residues involved in vitamin B12 binding and enzyme function.

This grade 1A SERC-supported position, tenable for three years, has a starting salary of £7,520 (under review) including USS benefits.

Applicants with suitable experience in recombinant DNA technology should send a curriculum vitae and the names of two referees to Dr Robert E Glass, Department of Biochemistry, Medical School, Queen's Medical Centre, Nottingham NG7 2UH (telephone: 0602-70011 Ext 3395) from whom further information may be obtained.

(7069)A

UNIVERSITY COLLEGE CARDIFF

DEPARTMENT OF GEOLOGY LECTURESHIP IN SEDIMENTOLOGY

Applications are invited for the above post. It involves broad teaching duties in the context of Geology degree schemes, but carries special responsibility for undergraduate and graduate courses in sedimentology. The successful candidate will be expected to develop departmental facilities and research activities in sedimentology. Preference will be given to applicants who have a strong research interest in clastic sedimentology. Salary range £7,520 — £14,925 per annum. Duties to commence 1st October, 1985 or as soon as possible thereafter.

Applications (two copies), together with the names and addresses of two referees, should be forwarded to the Vice-Principal (Administration) and Registrar, University College, PO Box 78, Cardiff CF1 1XL. From whom further particulars will be available. Closing date 8th August, 1985. Ref 2980. (7064)A

Faculty Position available, tenure-track, in molecular genetics, with a special interest in membranes. Applicants should have research background in recombinant DNA technology (preferably also in somatic cell genetics) and working knowledge of membrane proteins. M.D. and/or Ph.D. in appropriate field required. Successful candidate will hold tenure-track appointment (Assistant or Associate Professor, depending upon qualifications) in Department of Physiology and Molecular Biophysics at Baylor College of Medicine. Joint appointments in relevant academic departments also possible. Laboratory space and basic equipment provided and initial laboratory support available. Successful candidate will devote majority of his/her effort to establishing and maintaining an independent research program related to the main interests of the Department. The Department is building a strong program in membrane biophysics with expertise in electrophysiology, membrane biochemistry, reconstitution, and modelling. **Send vita, a brief description of past and present research experience, and names of three or more references to**

Dr. A. M. Brown, Incoming Chairman, Physiology and Molecular Biophysics Department



**Baylor College of Medicine,
One Baylor Plaza,
Houston, Texas 77030.**

An Equal Opportunity A/A Employer

(NW27)A

SEMINARS & SYMPOSIA

HUMAN GENETICS 1985

A Symposium organized by the Committee on Genetic Experimentation (COGENE) of the International Council of Scientific Unions (ICSU) and co-sponsored by the Institut National de la Santé et de la Recherche Médicale (INSERM) and the Association pour la Recherche contre le Cancer (ARC), will take place in Paris between October 2-4, 1985. The Program of the Symposium has been arranged by an Organizing Committee formed by G. Bernardi, F. Gros (co-chairmen), S. N. Cohen, W. Mommaerts, R. Monier, A. Skalka and R. Williamson. The five sessions of the Symposium will deal with:

- (1) The human genome
- (2) Oncogenes and cancer
- (3) Gene control and development
- (4) Genetic diseases
- (5) Perspectives in diagnosis and gene therapy

Session chairmen and speakers comprise: W. Arber (Basle), M. Bassendine (Edinburgh), G. Bernardi (Paris), M. Buckingham (Paris), A. de la Chapelle (Helsinki), D. Cohen (Paris), C. Croce (Philadelphia), J. Dausset (Paris), C. de Duve (Bruxelles), L. Ernster (Stockholm), G. Felsenfeld (Bethesda), F. Gros (Paris), F. Jacob (Paris), U. Littauer (Rehovot), L. Luzzatto (London), J.-L. Mandel (Strasbourg), J. O'D. McGhee (Oxford), W. Mommaerts (UCLA), R. Perry (Philadelphia), U. Pettersson (Uppsala), E. de Robertis (Basle), J. Rosa (Créteil), D. Stehelin (Lille), J. G. Sutcliffe (La Jolla), D. Weatherall (Oxford) and R. Williamson (London).

Attendance at the Meeting will be free of charge; a pre-registration is required and can be obtained by writing to Dr G. Bernardi, Laboratoire de Génétique Moléculaire, Institut Jacques Monod, 2 Place Jussieu, 75005 Paris (France).

(W2030)M

I² WORLD CONFERENCE ON INFLAMMATION

ANTIRHEUMATICS, ANALGESICS, IMMUNOMODULATORS

Montecarlo, Principality of Monaco - March 19/22, 1986

- 1) New Antiinflammatory Drugs
- 2) New Analgesics
- 3) New Immunomodulators
- 4) New Cortisone Derivatives
- 5) Inflammatory Experimental Models for Drug Evaluation
- 6) Recent Advances for Clinical Evaluation of New Anti-rheumatic Analgesic and Immunomodulator Drugs
- 7) Arachidonate Metabolites in Inflammation - Prostaglandin Activators and Inhibitors - Lipoxigenase Activators and Inhibitors - Phospholipase A₂ - Crotoxin
- 8) Plasminogen Activators - Connective Tissue Activating Peptides - Proteases and Protease Inhibitors
- 9) Mast Cells - Histamine - Kinins - Serotonin - P Substance
- 10) PAF in Pharmacotherapy - PAF Activators and Inhibitors - PAF in Immunological Reactions
- 11) Platelet Receptors - Platelet Derived Mediators - Platelets and Calcium Mobilization - Pharmacology and Clinical Pharmacology of Platelet Inhibitory Drugs
- 12) Free Radical Scavengers - Superoxides and Lipoperoxides
- 13) Fever and Antipyretic
- 14) Immunotherapy
- 15) Immunomodulators of Microbial Origin - New Synthetic Immunomodulators - Muramyl Dipeptides Derivatives - Inosine Derivatives - Thiolic Derivatives - Cyclosporin A - Pyrimidones Derivatives - Thymic Factors
- 16) Interleukines - Interferon - Interleukines and Interferon in Tumor Control - Cytokines in Arthritis
- 17) Methods for Evaluation of New Immunomodulators
- 18) Antibiotic Interaction in Immune Response
- 19) Natural Synthetic and Semisynthetic Opioids - Opioid Receptors - Opioids Association - Opioids and ACE System
- 20) Pain Control - Calcitonin - Somatostatin
- 21) Burns Therapy
- 22) Clinical Trials on New and Old Antirheumatic, Antiarthritic Analgesic and Immunomodulator Drugs
- 23) Wound Healing
- 24) Side Effects and Toxic Effects of Antiinflammatory, Analgesic and Immunomodulator Drugs

Round Tables and Satellite Symposia on special topics are also planned

SUBMISSION OF PAPERS: Please submit abstracts in English not exceeding 250 words, not later than September 30, 1985. Five Poster Sessions are also planned

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A. Bertelli (Pisa, Italy)
J.C. Houch (Seattle, USA)
Y. Mizushima (Tokyo, Japan)

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Prof. Aldo Bertelli
Institute of Pharmacology - University of Pisa
Via Roma, 55 - 56100 Pisa (Italy)
Ph 050/22312-46009 - Telex 590035 UNIV PI I

ORGANIZING SECRETARIAT

ALM s.r.l.
Via Lattuada, 26 - 20135 Milano
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Telefax 2844035

(W2029)C

THE UNIVERSITY OF LEEDS MSc in CARDIOVASCULAR STUDIES

A one year full-time course which provides a training for scientific investigations into the human and other mammalian cardiovascular systems

The course consists of lectures, tutorials and laboratory work which provide experience in. Isolated tissue studies; Mammalian systems physiology; Cardiovascular investigation in man; Exercise physiology, Pathophysiology of the cardiovascular system, Neuronal control of the circulation, Respiratory physiology; Advanced studies in cardiology

A major component of the course is a three month research project.

The course is open to graduates in medicine, physiology and related disciplines and commences 1 October each year. For further details write as soon as possible to the Department of Cardiovascular Studies, The University, Leeds LS2 9JT. (7072)C

ASSISTANTSHIPS

CAMBRIDGESHIRE COLLEGE OF ARTS AND TECHNOLOGY

(Equal Opportunity Employer)

DEPARTMENT OF SCIENCE
RESEARCH
ASSISTANTSHIPS (LEA)

Research Assistants (3) are required from 1st September, or as soon as possible thereafter, to carry out supervised research in the following areas:

1. Radionuclide and rare earth element geochemistry
2. Plant genetic engineering
3. Mammalian semiochemistry
4. Insect-plant biochemical interactions (use of micro-electrochemical sensors)

Applicants should possess a good honours degree, or MSc, in any appropriate discipline, and will be expected to register for the MPhil/PhD degree of the CNA. They may be required to carry out a limited amount of teaching. The posts are for three years in the first instance, with the possibility of extension for up to one year.

Please state the post(s) for which application is made

Salary Scale: £5,181 — £5,910 (award pending) Candidates with a higher degree start at £5,424.

Details and forms, to be returned by 26th July, from Administrative Assistant, Department of Science, CCAT, East Road, Cambridge CB1 1PT, Tel: (0223) 352992. (7058)P

FELLOWSHIPS

THE AUSTRALIAN NATIONAL UNIVERSITY

Faculty of Science

DEPARTMENT OF BOTANY

VISITING FELLOWSHIPS

Applications are invited for Visiting Fellowships in the Department of Botany, Faculty of Science, to be taken up during the next two years. These awards are intended to assist scientists working in any field of plant science, including genetics and microbiology, to spend a period of between 3 and 12 months carrying out research and/or preparing material for publication. The work may, but need not, involve collaboration with a member of the Botany Department. Visiting Fellows are expected to work in the Botany Department for most of the period of their appointment, during which time office/laboratory accommodation and adequate facilities for a agreed programme will be available. University housing at prevailing rates may also be provided.

Two types of Visiting Fellowship are offered: (1) Alexander von Humboldt — Australian National University Visiting Fellowships, for applicants based outside Australia and New Zealand, of value \$A4,000; (2) Visiting Fellowships for applicants based in Australia or New Zealand, of value \$A1,000. The grant are intended to assist towards travel costs and other expenses.

Applicants, who must have demonstrated research ability, should provide a curriculum vitae and list of publications together with an informative account of their proposed work as a Visiting Fellow and a statement on the proposed timing of the visit; applications quoting Ref FS 20.6.1, should reach the Acting Registrar of the University, GPC Box 4, Canberra, ACT 2601, Australia, by 30 September 1985. Further information on these Fellowships and on Botany Department facilities, staff, courses and research interests can be obtained from Professor J. Warren-Wilson, Head of the Department of Botany, Australian National University (062) 492866.

Further particulars also available from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square London WC1H 0PF. The University is an equal opportunity employer. (W2027)E

POSTDOCTORAL FELLOWSHIP IN MOLECULAR NEUROBIOLOGY

Friedrich Miescher-Institut,
Basel, Switzerland.

Applications are invited for an internally funded fellowship, of three years maximum duration, to join an established team working on the expression of neuron-specific proteins in developing brain using recombinant DNA techniques. A good training and demonstrated ability in molecular biology are essential. The Institute provides outstanding facilities in a purely research environment. Inquiries should be addressed to Dr A. Matus, Friedrich Miescher-Institut, P.O. Box 2543, CH-4002 Basel, Switzerland. (W2014)E

continued on page 19

UNIVERSITY OF WARWICK

POST-DOCTORAL
RESEARCH FELLOWSHIP
BIOLOGICAL SCIENCES

Applications are invited for an SRC-funded post-doctoral research fellowship to investigate the molecular mechanism of extracellular enzyme export in *Erwinia trovora*. The project will involve enzyme (pectinases, cellulases and proteases) localization techniques, mutant analysis, gene cloning, sequencing and gene product identification. Applicants should have, or shortly expect to have, a PhD, and candidates with skills in molecular biology and/or enzymology will be referred.

The position is for 3 years and is available from 1st October, 1985. Salary on the 1A scale: £7,520 to £12,150 pa. Applicants should send a CV (including a full list of publications) with the names and addresses of two referees, to the Executive Officer, Department of Biological Sciences, University of Warwick, Coventry, CV4 7AL, quoting reference GPCS/NAR. Additional information can be obtained from Dr G P Almond on ext 6098.

Closing date: 26th July, 1985
(7085)E

POSTDOCTORAL RESEARCH FELLOWSHIPS

The LBI-Basic Research Program emphasizes research on eukaryotic gene expression and regulation, molecular oncology, prokaryote and eukaryote genetics, protein chemistry, and chemical carcinogenesis. The senior staff members and their research interests are as follows:

George F. Vande Woude: molecular basis of neoplastic transformation

Mariano Barbacid: oncogenes in human and carcinogen-induced animal tumors

Stephen Hughes: retroviral vectors, expression of cytoskeletal genes

James N. Ihle: regulation of hematopoietic/lymphoid differentiation, molecular mechanisms of leukemogenesis

George N. Pavlakis: eukaryotic gene regulation, molecular mechanisms of cell transformation

Stephen Oroszlan: immunochemistry and protein chemistry of retroviruses, structure and function of retroviral gene products

Alan Rein: retroviral genetics, functional analysis of retroviral genes using natural and synthetic mutants

Nancy Rice: genomic organization of endogenous and exogenous retroviruses, transforming genes, cloning, DNA sequencing, expression

Maurice Cohen: molecular biology of human retroviruses, developmental regulation of expression

William Lijinsky: environmental carcinogenesis, mechanisms of carcinogenesis by nitrosamines and related compounds

Anthony Dipple: carcinogen-DNA interactions, aromatic polycyclic hydrocarbon metabolism

Christopher Michejda: organic chemistry and biochemistry of carcinogen activation

Jeffrey Strathern: cell type regulation, genome rearrangement in yeast

Stuart Austin: regulation of replication, segregation of and gene expression by autonomous replicons

Neal Copeland: molecular genetics of murine leukemogenesis, developmental genetics, transposable elements and retroviruses

Nancy Jenkins: insertional mutagenesis by retroviral DNAs, transgenic mice, molecular biology of mouse development

For further information, contact Dr. Maurice L. Guss, LBI-Basic Research Program, NCI-Frederick Cancer Research Facility, P.O. Box B, Frederick, Maryland 21701-1013



LITTON BIONETICS, INC. - BASIC RESEARCH PROGRAM
NATIONAL CANCER INSTITUTE - FREDERICK CANCER RESEARCH FACILITY

An equal opportunity/affirmative action employer M/F/H/V

(NW28)E

THE ANIMAL VIRUS RESEARCH INSTITUTE
POST-DOCTORAL FELLOWSHIP IN
MOLECULAR BIOLOGY

(Funded by The Overseas Development Administration)

Applications are invited for a 4 year fellowship (beginning October 1st 1985) to work on analysis of the genome of capripox viruses and their development for use as a eukaryotic vector system.

The successful candidate will work with Dr Donald Black in The Division of Molecular Biology. He/she should have a degree in Biochemistry or a related subject and a PhD. Experience with expression systems and eukaryotic gene analysis would be an advantage.

The salary will be in the scale £7,788 to £10,541 (Higher Scientific Officer Grade). Sick leave and non-contributory superannuation schemes, 22 days annual leave. Accommodation may be available. The AVRI is an equal opportunity employer.



Application to include a curriculum vitae and the names of two referees should be sent as soon as possible to the Secretary, Animal Virus Research Institute, Ash Road, Pirbright, Woking, Surrey GU24 0NF. Tel. 0483 232441. (7010)E

IMMUNOLOGY

POST-DOCTORAL FELLOWSHIP

The Veterinary Infectious Disease Organization (VIDO), a dynamic and growing research institute located at the University of Saskatchewan is seeking applications for a post-doctoral position. The successful applicant will join a group investigating the biology and immunological properties of bovine recombinant recombinant α and γ interferon. Although the applicant will be primarily involved with *in vitro* studies, there will be ample opportunity to participate in *in vivo* trials as well. The virus models being investigated are enteric (rotavirus and coronavirus) and respiratory (bovine herpesvirus-1) infections in cattle.

Expertise in modern cellular immunological techniques and a PhD in immunology is required.

Salary range \$22,000 — \$25,000. Salary commensurate with experience. Send résumé and telephone numbers of three referees to:

Dr Lorne A Babiuk
Associate Director (Research)
Veterinary Infectious Disease Organization
124 Veterinary Road
Saskatoon, Saskatchewan
S7N 0W0.

(NW21)C

E M B O

EUROPEAN MOLECULAR BIOLOGY ORGANIZATION
SHORT TERM FELLOWSHIPS
in molecular biology

The European Molecular Biology Organisation awards, to scientists working in Europe and Israel in the field of molecular biology and allied disciplines, short term fellowships of one week up to three months duration. The fellowships are to support collaborative research between laboratories in different countries and provide a travel grant and subsistence allowance. Applications may be made at any time and are decided upon soon after the receipt of application.

Applications for exchanges between laboratories within any one country cannot be considered. Fellowships involving transatlantic travel are awarded only in extremely exceptional circumstances.

Application forms and further details may be obtained from Dr J. Tooze, Executive Secretary, European Molecular Biology Organization, 69 Heidelberg 1, Postfach 102240, F.R.G. (W1577)E

ASSISTANTSHIPS CONTINUED

TEESSIDE POLYTECHNIC

Department of Chemistry

LEA RESEARCH ASSISTANTSHIP
IN SYNTHETIC ORGANIC CHEMISTRY

Applications are invited from suitable qualified candidates for an LEA Research Assistantship in Organic Chemistry.

The programme, leading to the degrees of MPhil and/or PhD is concerned with the synthesis and biological evaluation of potential anti-tumour agents, to commence in September 1985 for 3 years only. Candidates will be expected to have or expect to obtain a first or good second class honours degree or equivalent in Chemistry and have interests in total synthesis.

Salary, which includes payment for up to 6 hours per week teaching assignment is £5,910 — £6,657 per annum.

Closing date for applications: 31 July 1985.

An Equal Opportunity Employer

Further particulars and application forms from: The Personnel Section, Teesside Polytechnic, Borough Road, Middlesbrough, Cleveland TS1 3BA, Telephone: (0642) 218121, Extension 4114. (7070)P

CONGRESS

INTERNATIONAL SOCIETY OF DEVELOPMENTAL BIOLOGISTS
Announces theTENTH INTERNATIONAL CONGRESS OF ISDB
"NEW DISCOVERIES AND TECHNOLOGIES"
AUGUST 4-9, 1985The Westin Bonaventure Hotel
Los Angeles, California, USA

PARTICIPANTS

Peter Albersheim, Merton Bernfield, Hans Bode, James Bonner, Jeremy Brockes, Donald Brown, Arnold Caplan, Pierre Chambon, Thomas Cline, Jonathan Cooke, Gerald Edelman, Robert Edwards, Judah Folkman, Scott Fraser, Vernon French, Richard Gardner, Walter Gehring, Benjamin Geiger, Norton Gilula, Paul Green, Suresh Goel, Clifford Grobstein, Elizabeth Hay, Ross Hodgetts, Ernest Jaworski, Howard Jones, Jr., Yoshihiro Kato, Klaus Kratochwil, Elias Lazarides, Nicole Le Dourain, Edward Lewis, Anne McLaren, Alberton Monroy, Aron Moscona, Drew Noden, Christiane Nusslein-Volhard, Daphne Osborne, Masukichi Okada, Richard Palmiter, Martin Raff, William Rutter, Kenneth Ryan, Gordon Sato, Lauri Saxén, Jozef St Schell, Robert Schimke, Howard Schneiderman, David Schubert, Yingshien Shih, Allan Spradling, Timothy Stewart, Masatoshi Takeichi, Jean Paul Thiery, Robert Trelstad, Victor Vacquier, Serger Vassetzky, Stephen Wachtel, Lewis Wolpert, Savio Woo, Carl Wood

SPECIAL LECTURER: DR. STEPHEN J. GOULD
"WHAT DEVELOPMENT CAN TEACH ABOUT EVOLUTION"

OFFICIAL ADDRESS FOR REGISTRATION
Harold C. Slavkin, Chairperson 1985
ISDB ORGANIZING COMMITTEE
University of Southern California
University Park, GER 314-MC 0191
Los Angeles, California 90089-0191
(213) 743-6095 or 743-6585

(NW1920)Q

STUDENTSHIPS

INSTITUTE OF
PHYSIOLOGYUNIVERSITY OF GLASGOW
RESEARCH STUDENTSHIP

Leading to Ph.D. and commencing 1st October. The project, which is supported by the Foundation for Age Research, will investigate ageing changes in the human fovea by histological, electrophysiological and psychophysical methods. The appointee should possess a class 2(1) degree in a relevant subject and will be part of an active vision group with close clinical contacts. Applications (curriculum vitae with 2 referees) to Dr J. D. Morrison (Tel. 041 339 8855, ext. 499) as soon as possible. (7098)F

THE SCHOOL OF
PHARMACYUniversity of London
RESEARCH STUDENTSHIP
IN CHEMISTRY

A SERC case research studentship, in conjunction with Unilever plc, is available from October 1985 leading to a higher degree. The project involves the measurement of diffusion coefficients of macromolecular materials using classical and elastic light scattering techniques.

Applicants should have, or expect to obtain a good honours degree in Pharmacy, Chemistry, Biophysics or Physics.

Interested persons should contact: Dr R Fleming, The School of Pharmacy, 29/39 Brunswick Square, London, WC1N 1AX as soon as possible (7079)F

ROTHAMSTED
EXPERIMENTAL STATION
HARPENDEN,
HERTS. AL5 2JQSTUDENTSHIP: GENETIC
MANIPULATION IN
POTATO

Applications are invited for a Ph.D. Studentship at Rothamsted Experimental Station to study expression of RIT-DNA genes in potato and their effect on potato development. The project will involve molecular and cell biological techniques and the use of Agrobacterium to obtain transformed plants.

Candidates should hold a First or Upper Second Class Degree in Biology, Biochemistry or related subject.

Applications giving full c.v., the names of two referees and quoting Ref. 598 should be sent, not later than 30 July 1985, to Dr G Ooms, Biochemistry Department, Rothamsted Experimental Station, Harpenden, Herts. AL5 2JQ.

We are equal opportunities employers. (7095)F

UNIVERSITY OF
LEICESTER
POSTGRADUATE
RESEARCH IN PHYSICS
Ph.D.

Three S.E.R.C. studentships are available leading to the degree of Ph.D. in the following areas:

Theoretical Physics
(Hot electron dynamics in Quantum wells)
Ionospheric Physics

UNIVERSITY COLLEGE
CARDIFFDEPARTMENT OF PHYSIOLOGY
PhD STUDENTSHIP

Applications are invited for a Research Studentship in a project concerned either with: a) Synaptic interactions in the central visual system b) Modulation of sensory transmission through thalamic relay nuclei

The projects will take place in well equipped MRC funded laboratories and will offer access to a wide range of the latest techniques. Applicants should have a First or Upper Second Honours Degree in a relevant scientific subject. Those interested should apply to Professor A M Sillito, Department of Physiology, University College, PO Box 78, Cardiff CF1 1XL, enclosing a brief Curriculum Vitae and the names and addresses of two referees. Informal enquiries can be made to Professor Sillito by telephone on 0222 44211 Ext 2062, and potential applicants will be welcome to visit the laboratories (7025)F

CASE STUDENTSHIP:

Spectroscopic Studies of
Adsorbed Surfactant Molecules

This project, sponsored by Unilever Research, is aimed at the detailed study of the structure, arrangement and interactions of surfactant molecules chemisorbed on a variety of substrates ranging from metals to fabrics. Infrared and Raman studies will be combined with absorption isotherm and esr measurements to investigate the ways in which detergent molecules adhere to industrially relevant materials. Both molecule-molecule and molecule-substrate interactions are important and will be studied as a function of chemical (head group) properties, coverage and method of deposition. The work will be jointly supervised by Dr Jack Yarwood (at Durham) and Dr Kay Fox (at Unilever, Port Sunlight). The student will spend at least 4 weeks per year working at Port Sunlight and will receive £400 per annum (subject to satisfactory reports) in addition to the usual SERC grant.

Applications, naming a referee, from those who have (or expect to have) at least an upper 2nd class degree in chemistry should be sent as soon as possible to Dr J Yarwood, Department of Chemistry, University of Durham, South Road, Durham, DH1 3LE. (7066)F

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Those wishing to develop proposals should write for further details to Mr P. F. Slack, Ministry of Agriculture, Fisheries and Food, Room 117, Great Westminster House, Horseferry Road, London SW1P 2AE.

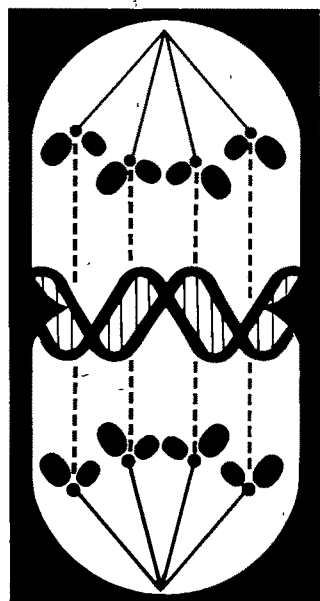
The closing date for submissions will be Monday, September 16th, 1985. (7089)X

APPTS. WTD

BIOCHEMIST: PhD (CSIR) 197: (biomedical mechanism of 'contractile' action), 10 years' professional experience includes 7½ years research and teaching clinical aspects of Biochemistry, 2½ years in clinical diagnostic labs as biochemist director; publications. Presently Associate Professor in Medical School. Seeks similar appointment. Write Box W2022 c/o Nature Classified, 4 Little Essex Street, London WC2R 3LF. (W2022)B

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J. Gerhart (U. of California at Berkeley)	Early development—Xenopus
C. Nüsslein-Volhard (Tubingen)	Early development— <i>Drosophila</i>
C. Goodman (Stanford)	Development of invertebrate neural systems
H.R. Horvitz (MIT)	Caenorhabditis

DAY 2: TUESDAY, OCTOBER 8

CELLS

Speakers	Topics
M. Davis (Stanford)	T-cell receptors

U. Storb (U. of Washington at Seattle)	B cells
J.M. Bishop (U. of California at San Francisco)	Oncogenes
M. Ptashne (Harvard)	Regulatory proteins
I. Herskowitz (U. of California at San Francisco)	Yeast development
E. Jaworski Monsanto	Plant transformation

DAY 3: WEDNESDAY, OCTOBER 9

GENES

Speakers	Topics
R. Palmiter (U. of Washington at Seattle)	Transgenic mice
D. Hogness (Stanford)	<i>Drosophila</i> homoeotic genes
W. McGinnis (Yale)	Mammalian homoeotic genes
T. Friedmann (U. of California at San Diego)	Human therapy
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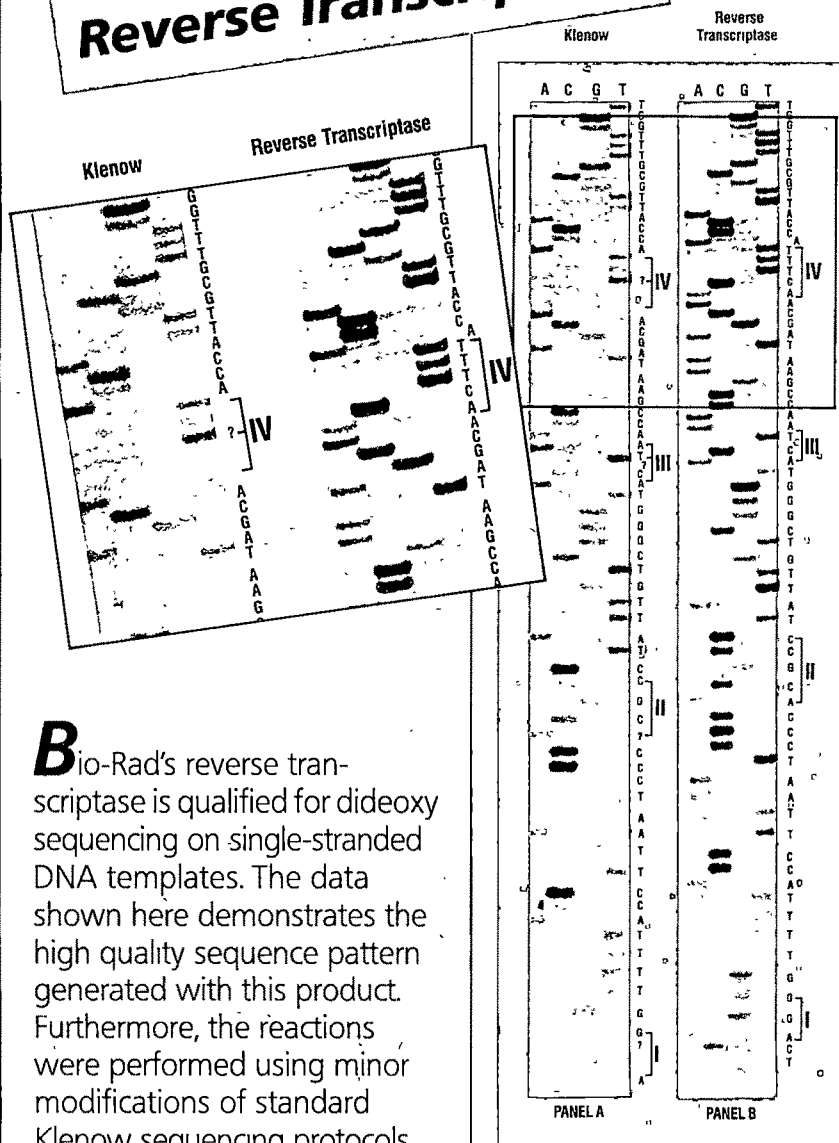
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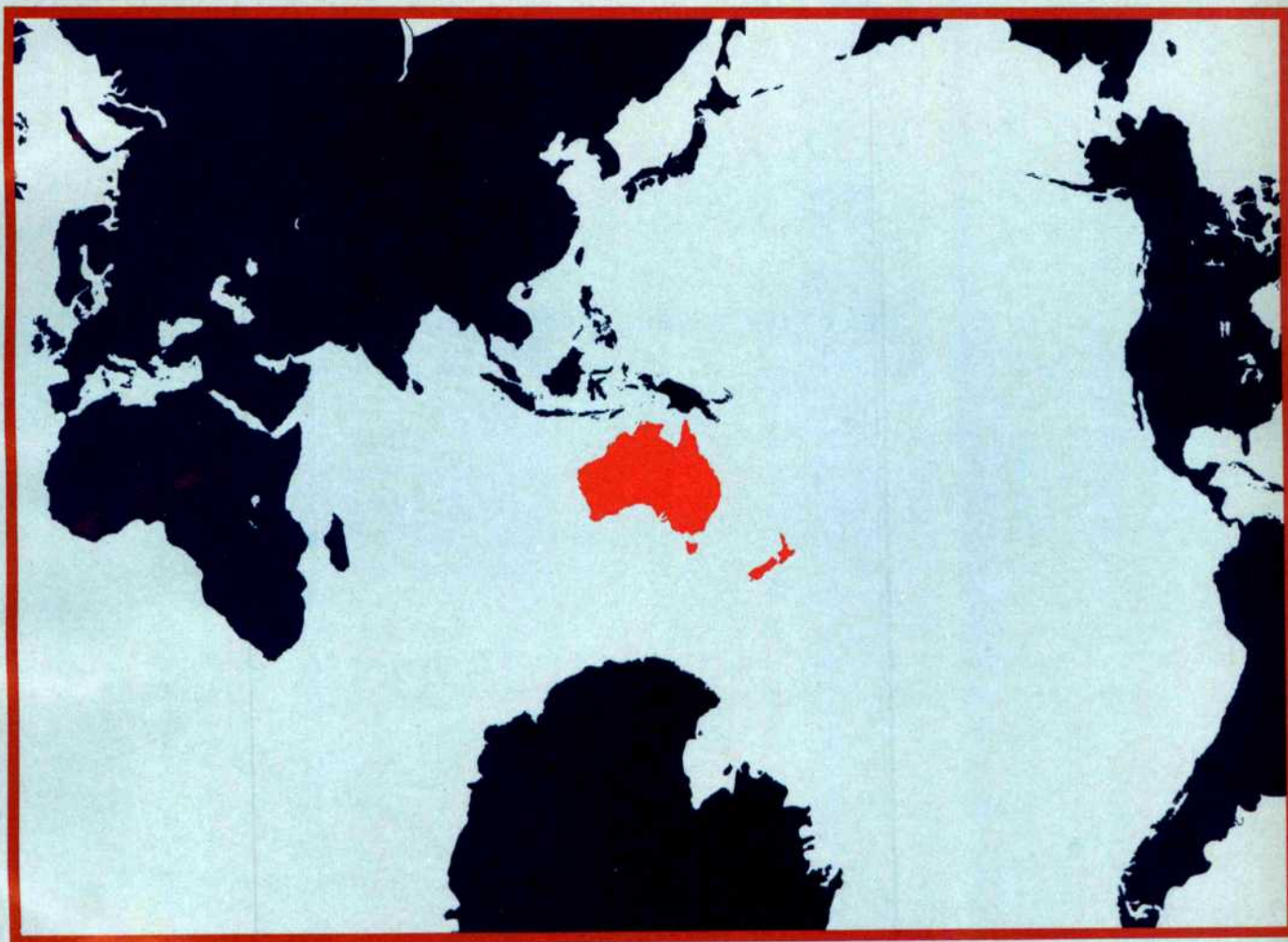
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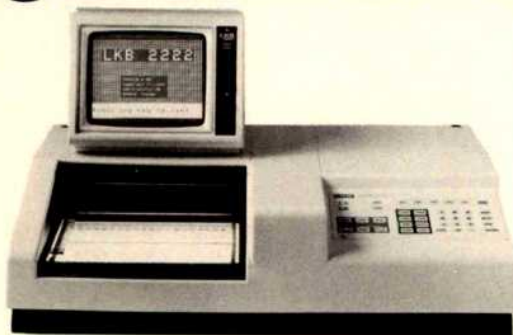
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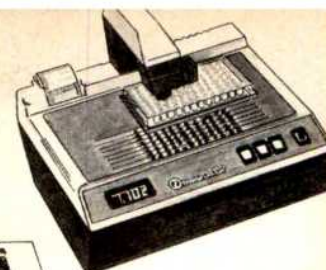
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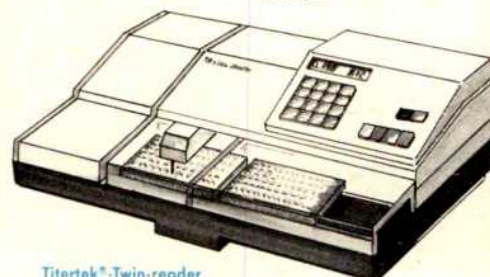
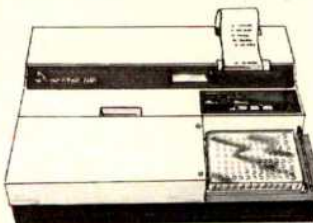
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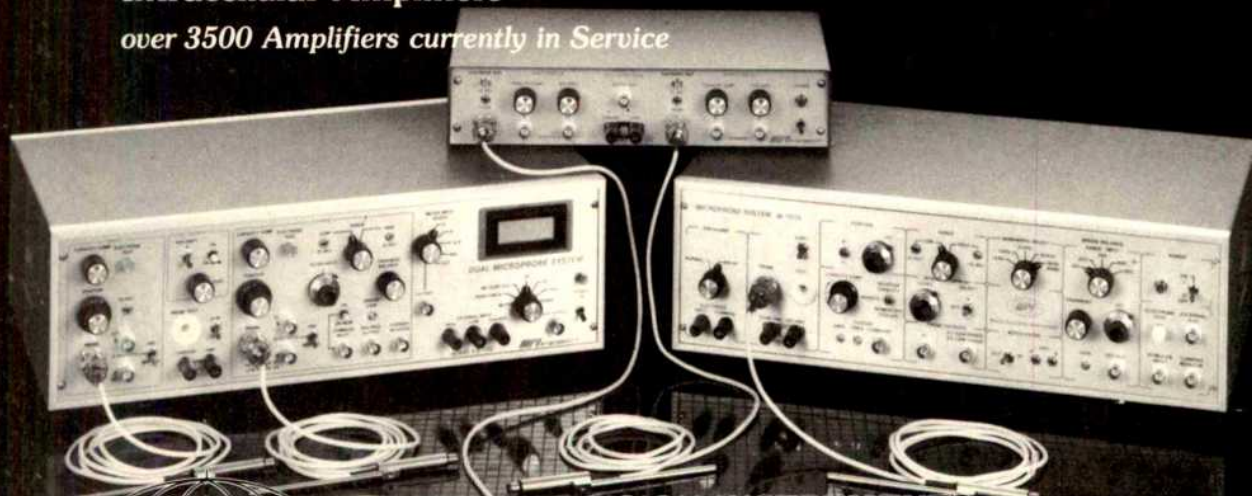
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SCIENCE IN AUSTRALASIA

The world as seen relative to Australia. The projection is Mercator's cylindrical, centred on Mid-Australia (25° S, 134° E) with 360° azimuth coverage and a half-height of 70° of latitude. In this issue the state of science in Australia and New Zealand is reviewed on pages 185–208. (Map courtesy J. Waldoock, Leicester University.)

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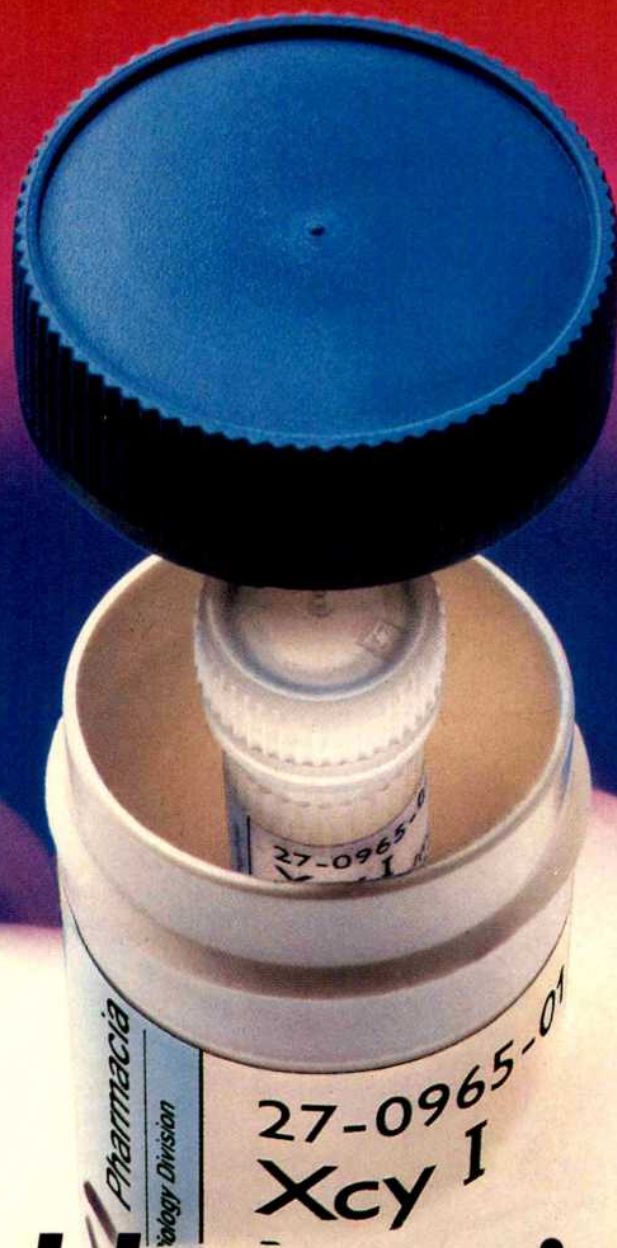
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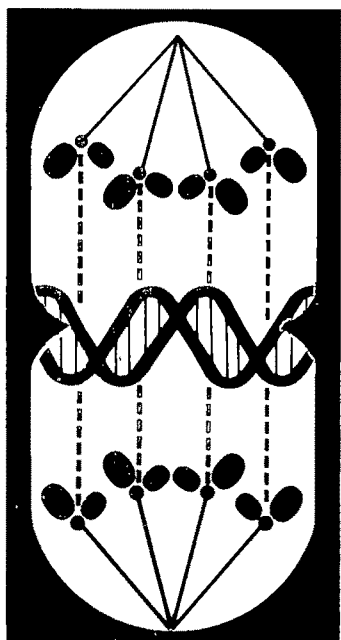
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PROGRAM SCHEDULE*

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SYSTEMS

Speakers

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(Research Institute
of Scripps Clinic)

J. Gerhart
(U. of California
at Berkeley)

C. Nusslein-Volhard
(Tübingen)

C. Goodman
(Stanford)

H.R. Horvitz
(MIT)

Topics

Vertebrate neural
systems

Early development—
Xenopus

Early development—
Drosophila

Development of
invertebrate
neural systems

Caenorhabditis

DAY 2: TUESDAY, OCTOBER 8

CELLS

Speakers

M. Davis
(Stanford)

U. Storb
(U. of Washington
at Seattle)

J.M. Bishop
(U. of California
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M. Ptashne
(Harvard)

I. Herskowitz
(U. of California
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E. Jaworski
Monsanto

Topics

T-cell
receptors

B cells

Oncogenes

Regulatory
proteins

Yeast
development

Plant
transformation

DAY 3: WEDNESDAY, OCTOBER 9

GENES

Speakers

R. Palmiter
(U. of Washington
at Seattle)

D. Hogness
(Stanford)

W. McGinnis
(Yale)

T. Friedmann
(U. of California
at San Diego)

Topics

Transgenic mice

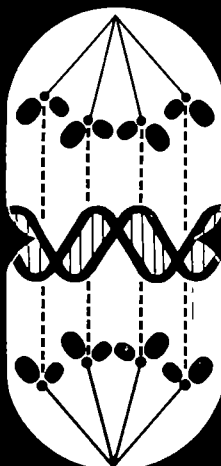
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Norman Borlaug, CIMMYT, Mexico City and Professor, Dept. of Soil and Crop Science, Texas A&M

JANUARY 20—AFTERNOON

Seminar in Molecular Biology

David Goeddel, Co-Chairman

Richard Flavell, Co-Chairman

Budd Colby, Director, Technology Development, Triton Biosciences

Seminar in Agricultural Biotechnology

Norman Borlaug, Chairman

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David Evans, Vice President for Research, IMA Plant Technology, Inc

Charles Arntzen, Associate Director of Central Research and Development, E. I. du Pont de Nemours and Co.

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Commercial Production Processes

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Malcolm Lilly, Professor of Chemistry and Biochemical Engineering, University College, London

Robert Herschberg, Department of Process Development, Genentech

Yukio Sugino, Director, Biotechnology Labs, Akeda Chemical, Japan

Ephraim Katchalski-Katzir, Professor of Biophysics and Biotechnology, Tel Aviv University, Israel

JANUARY 21—AFTERNOON

Seminar in Scale Up

Malcolm Lilly, Co-Chairman

Robert Herschberg, Co-Chairman

Garry Buckland, Director, Computerized Pilot Plant, Merck, Sharp, & Dohme

Stanley Tarnowski, Research Section Chief, Dept. of Bioseparations, Hoffmann-La Roche

Seminar in Fermentation

Yukio Sugino, Chairman

Arnold Demain, Director of Laboratory of Fermentation and Microbiology, MIT

Behzad Khosrovi, Senior Director, Process and Product Development, Cetus Corp

William Tolbert, Research Fellow, Monsanto

Seminar in Immobilization & Biocatalysis

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Emil Thomas Kaiser, Haggerty Professor, Rockefeller University

George Poste, Vice President and Director of R&D, Smith Kline & French

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Pablo Valenzuela, Vice President of Research, Chiron Corp

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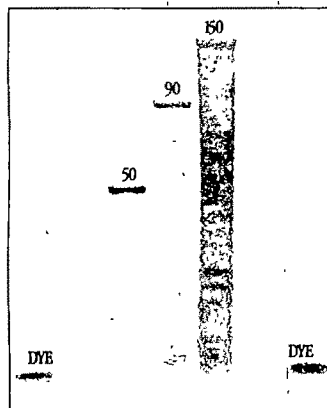
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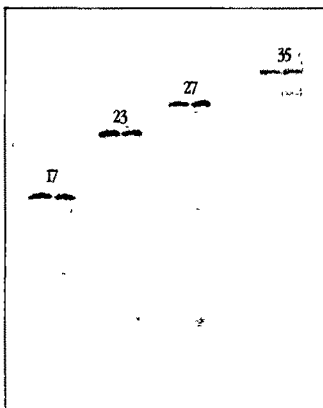


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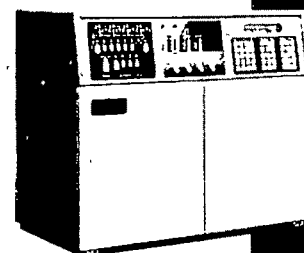
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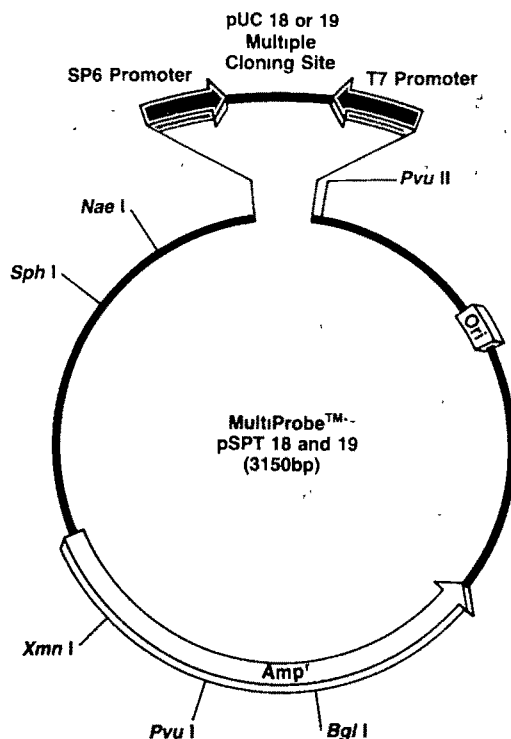
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What price depleted uranium?

A few weeks before the third review conference of the Non-Proliferation Treaty (NPT), it is disturbing that large quantities of depleted uranium have been sold without safeguards.

A MONTH from now, the signatories of the Non-Proliferation Treaty (NPT) will be gathering at Geneva for a conference to review the terms of the treaty. If this conference follows the two earlier meetings of its kind (1975 and 1980), there will be diplomatic ructions. Even though, by all accounts, preparations have been carried out more smoothly than those for previous conferences, it is probably asking too much of the non-nuclear powers that they do not question the precious little progress that the nuclear powers have made towards their promise under the treaty to negotiate measures of strategic arms control "in good faith". On past form, but also with some reason, non-nuclear powers will argue that more should have been done to spread the civil benefits of nuclear technology. But what will they, and even the nuclear powers which have signed the treaty, make of the puzzling news last week that companies in Luxembourg and Belgium have supplied to Israel several tonnes of depleted uranium in the fissile isotope uranium-235, and that Euratom, Europe's own safeguards authority, was not informed?

It is tempting to think that uranium metal consisting primarily of uranium-238, a waste material in isotope separation plants, has no military significance. Since the first huge hydrogen bomb explosions on the Marshall Islands in the Pacific during the 1950s, it has been clear that ordinary uranium of any isotopic composition may be useful to those who would increase the yield of their explosions. To make a uranium fission-bomb, or a uranium trigger for a hydrogen bomb, it is of course necessary to rely on uranium-235, whence all the fuss about diffusion plants, centrifuges and, now, lasers as a means of separating this isotope from the two others that naturally occur. But in a hydrogen bomb, in which a thermonuclear explosion is ignited by a fission trigger, a surrounding shell of material consisting of heavy atomic nuclei will enhance efficiency by reflecting back into the explosion neutrons that would otherwise escape. Using uranium-238 as the external blanket has the further virtue, if that is the word, that a proportion of the material will undergo fission under the influence of fast neutrons from the explosion. All this was inferred, as long ago as 1955, by Dr Joseph Rotblat using data about the composition of fall-out from the Eniwetok explosion of 1952.

The fact that somebody in Israel has been buying depleted uranium does not, of course, imply that somebody in Israel is interested in making dirty hydrogen bombs. There are many other obvious uses for uranium-238, most of them deriving from its high density and its relative cheapness. But given the trouble that Israel has taken over the years to create the impression that it has gone a long way down the road to the manufacture of nuclear weapons, it is inevitable that suspicions will be aroused. Israel after all, is not a signatory of NPT. Whatever may be the use to which the uranium will be put, however, the fact remains that the sale from the Low Countries to Israel has been made

without the knowledge of Euratom, which has a special status in relation to the international safeguards system in that it has delegated authority from the International Atomic Energy Agency, NPT's inspector, to vouch for what happens within the European Communities to special nuclear materials. The issue has in the past been contentious: why, other members of NPT have asked, should European countries be able to make cosy relationships among themselves without direct invigilation from Vienna? Although it is not quite like that, in that Euratom safeguards are probably at least as demanding as those administered from Vienna, the members of NPT are likely to ask awkward questions about the lapse that came to light last week.

Confidentiality

What should be done? The members of Euratom have a vested interest in demonstrating that their hands are clean, and should do what they can in the next few weeks to show that that is the case. No harm would be done, even after the event, if the companies now found to have been selling depleted uranium on a commercial basis were to be asked, even compelled, by their governments to ask their customers what had become of it. Even if it should be discovered that the customers do not know, or will not say, what is being done with the depleted uranium, that will be useful information, which should of course be made available to the members of NPT. If it should turn out that the users can point to good reasons why they were buyers in the first place, and to the ways in which they are putting the material to good use, that too would be valuable information. The selling companies will no doubt say that such information, being commercial, is also confidential, but that is hardly an argument that should carry much weight; the sellers are, after all, those who have broken the law.

In the past few days, Euratom has clearly been hoping that the sale of the depleted uranium would be quietly forgotten; the names of the selling companies, for example, have not been disclosed. Similarly, no convincing explanation has been given of the source of the material sold, although there are only a few places from which it could have come (the diffusion plants in Britain and France, and the tripartite centrifuge enrichment operation involving Britain, the Netherlands and West Germany in Eurenco). It would be interesting, even significant, to know what Euratom has to say on this question, at the very least because a willingness to abide by the rules on the part of the legitimized uranium enrichment operations involving nuclear powers is an essential precondition of Euratom's chance of continuing to hold delegated authority for safeguarding what happens in Europe.

On the face of things, all this may seem irrelevant to what the NPT review conference will be considering at Geneva — a little local difficulty, so to speak. But that is far from the truth. NPT is already a sufficiently uneasy balance between powerful countries with nuclear weapons and often equally powerful countries that have agreed, for everybody's sake, that they will not go into the business. Transgressions, however small and accidental by the nuclear powers or their agents (those who sell their depleted uranium) could ironically be more unsettling than the errors (mostly of omission) at which the non-nuclear signatories now rail — in particular, the poverty of progress in arms control. □

Biological manuscripts

Contributors are reminded that, with the transfer of the Biological Sciences Editor to the Washington office, it will be helpful if they will in future send **four copies of all manuscripts** offered for publication, either (as at present) to London or Washington.

British politics

Mrs Thatcher asks for decisiveness

Mrs Margaret Thatcher, the British Prime Minister, remains convinced that the British research establishment would have no money problems if it managed its affairs properly. That was one of her opinions on the condition of British science to emerge from an interview last week.

Formally, since the government in 1982 rejected the House of Lords' advice that there should be a part-time science minister on the grounds that it already had a scientist as a prime minister, hers is the desk on which the buck stops. How much time can she spare for it?

Coordinating departments straddling different interests are a mistake, she says, the Cabinet Office in the person of the Chief Scientific Adviser, Sir Robin ("Robin has been wonderful") Nicholson does whatever administrative coordination is needed, and her role is that of a catalyst. She lists the open seminar in September 1983 of scientists and industrialists (with a sprinkling of politicians) of which she was the chairman, and several more recent initiatives. She seems especially pleased about the meeting earlier this year at which £42 million was extracted from the spending departments to assist the growth of numbers of science and technology students in higher education.

One outcome of the 1983 seminar, at which she promised that innovations supported by the public purse would not be offered for exploitation exclusively to the National Research Development Corporation (now part of the British Technology Group) has now been realized. Mrs Thatcher cannot understand why there was so much red tape to clear away. She had wanted individual scientists to benefit more directly from their innovations, but had had to struggle to get her way.

What of the other promise, in 1983, that British defence establishments would be made more open, both to universities and industry? There is some confusion. The defence people have produced an unsatisfactory list of civil spin-off from military research and have been asked to revise it. Fair play, the defence people now have a kind of entrepreneurs' organization of their own.

Wealth creation is the name of the game. Mrs Thatcher says that scientists must appreciate that money for research can come only by the creation of wealth, for which they have a responsibility. But things are not as bad as they seem. She could name a dozen teams of academics pointing in the right direction (and, indeed, she embarks on a list).

On the research councils, she insists that people must be prepared to put their

wishes in some order of priority. Complaints that first-class applications for research grants now often go unsupported are a sign that this is not happening. "People are not prepared to make decisions", she declares.

Mrs Thatcher is also offended at the way research councils administer their affairs. Applicants are asked to include three supporting opinions with their ap-



plications, but then "they send out for another three opinions", which is yet another sign that people are not prepared to take decisions. Why not instead give good institutions a quota of grants and let able professors decide who should get the money? Then the research councils could spend all their administration costs on research.

Reflectively, Mrs Thatcher wonders whether previous governments should not have been more "directional". During the university expansion of the 1960s, people may have been too conscious that the University Grants Committee is independent. The result was that too much of the expansion took place in the social sciences, not science and technology. And where else in the world does the government subsidize business schools?

Now she wants to see more centres of excellence in the universities. The trouble in the past has been that "we've not backed our judgement with money". This is what the University Grants Committee is now aiming at. (Sir Peter Swinnerton-Dyer, its chairman, is also "doing a very good job".) But Mrs Thatcher gives the impression that the recent green paper on higher education will not be her government's proudest achievement.

Mrs Thatcher also thinks it may have been a mistake to leave the judgement of what lines of research would be supported exclusively to the research councils. Earlier (as Secretary of State for Education and Science in the early 1970s), she had supported British high-energy physics. But now she wonders why the research councils are spending so much in this field when the same amount of money would

allow them to do much more elsewhere.

She has little sympathy for the research councils now short of research funds because of having to persuade people to retire early. They have not organized themselves for flexibility. "The worst thing you can do in research" is to put able people in narrow institutes; then "they work on tramlines".

The problem, as she sees it, is that of persuading the research councils to spend their money wisely. They have a "bounden duty" to take responsibility. Now, some people in the research establishment are beginning to do their duty. Sir Henry Chilver (director of the Cranfield Institute of Technology) is one, Dr John Ashworth (vice-chancellor of the University of Salford) is another. Mrs Thatcher contrasts these places, which are "creating jobs" with the other universities, in Manchester and Liverpool, where "we have these huge problems".

On the better exploitation of academic innovations, Mrs Thatcher agrees that industry may in the past have been at fault. People had to hawk their bright ideas around industry, often to no avail. But, she insists, the climate has now changed. It has taken a long time to get managers into a frame of mind to take decisions.

Mrs Thatcher praises the enterprise of the Victorian industrial revolution, acknowledges that one consequence may have been to divert large numbers of bright people into the administration of the old Empire, but says that, now, "the particular combination of genius that we have is not creating the wealth we need. Give me just one MIT", she pleads.

Mrs Thatcher's impatience is understandable, with the money supply once more out of balance. What will happen in the months ahead to the British government's research budget, and the recurrent grant for the universities, can only be a guess. But those who hope for extra funds probably hope in vain.

John Maddox

Informatik stretched

Bonn

THE Westdeutsche Rektorenkonferenz (WRK), official organization of the West German university rectors, has asked for tighter restriction of student access to courses in information sciences (*Informatik*). At their plenary meeting last week, the rectors pointed out that these new courses are overloaded by 200 per cent.

The president of WRK, Theodor Berchem, criticized the decision of the *Länder* (regional governments) to keep these courses open to general access. Part of the trouble is that the number of qualified professors for information sciences in West Germany is too small to cope with the demand. About 50 vacancies have not been filled, and for the planned 4,000 beginners (instead of 2,400 now), 100 additional positions for professors are needed.

Jürgen Neffe

Eureka

France optimistic on meetings

BRITAIN and France are coming closer together on issues of European science and technology policy, to judge by the impressions of M. Hubert Curien, the French Science Minister, of his flying visit to Britain last week.

"I was glad to see we all agree very closely on Eureka" said Curien, after an afternoon with Sir Keith Joseph, British Secretary of State for Education and Science, and Mr Geoffrey Pattie, Minister for Information Technology. Eureka, the French project to develop a series of new high-technology European products from supercomputers to genetically engineered seeds (see *Nature* 11 July p.97), is the subject of a 17-delegation European conference in Paris this week, and Curien was certainly interested during his British visit to test Britain's opinion after British isolation on issues of wider European integration at the recent summit in Milan. He left London "much more optimistic", and undismayed by reports that the British government is interested in Eureka only if it costs no new money. Anyhow, Eureka could start with only very meagre cash support, Curien intimated, but would not commit himself to precise figures for fear of prejudicing this week's meeting.

Out of the Paris meeting, Curien is seeking three things. First, an agreement on the principles of Eureka (that should be "no problem"); second, to produce a dossier of the interests of each country in the various "broad fields" of Eureka; and third and most difficult, to define a structure in which Eureka should be managed.

Curien was adamant last week that Eureka should not fall into the embrace of the European Commission. He would like to see a "very light" structure, although he said at a lunchtime meeting at the London-based Policy Studies Institute (PSI) that to avoid bureaucracy altogether could prove "idealistic". The European Commission could not manage Eureka because it had never produced products, and Eureka was strictly product-oriented, he said. The Commission's ESPRIT programme on stimulating cooperation among European companies in pre-competitive research in information technology is a success, but it stops at pre-competitive research — precisely because it is difficult at the Commission to reach unanimous agreement in the European Council (the Commission's political master) on real and potentially competitive products.

Curien is a past president of the French space agency CNES which designed, built and tested Ariane, Europe's space launcher, and naturally he sees the success of Ariane as a model for Eureka. The European Space Agency, which commissioned Ariane, had adopted in 1973 a policy of projects "à la carte". Ariane went ahead only because the French wanted it pas-

sionately: they paid two-thirds of the development cost, while Britain stood aside. "If it had been a mandatory programme we would have had no Ariane." Equally, Germany (with Italy) had shouldered most of the burden of developing Spacelab, and Britain had put 50 per cent support into the European Communications Satellite, ECS.

Such projects could not have taken place within the Commission structure, Curien felt, because of the present requirement for unanimity in the Council of Ministers. "The result is always support for the least offensive programme", Curien said, and it is "difficult to be ambitious" within the European Communities structure. (Ironically, the often ambitious Commission officials would certainly agree, faced as they often are by the immovable political obstacle of the Council of Ministers.)

Curien sees the future Eureka management as an "industrial" structure, focused on making things, which should be selected on technical merit and not political representation, Curien insists. And he

agreed with Sir Hermann Bondi, chairman of the PSI meeting, that Eureka will need a technical group "of a quality respected by industry". But "I don't want 17 representatives on everything", one from each state, says Curien.

Asked how Eureka projects would be chosen, Curien emphasized the need for specific, practical projects: "if we just say 'robotics' that would be useless"; but he was less insistent on the need to identify a market for each product. He seemed to be thinking largely along the lines of other major French government-led successes such as the high-speed train and nuclear power, and thus of government purchasers. He admitted, however, that both government and the free market must be considered.

Curien did not expect the Paris political meeting this week to define specific projects — that would be for working groups involving industry later on, once the political ground rules had been settled. However, he did hope to see "five or six" projects under way by the end of this year.

Robert Walgate

The 17 delegations should consist of the 12 European Community countries, plus Norway, Sweden, Austria, Switzerland and a delegation from the European Commission

US ASAT

Live test tactfully postponed

Washington

A PLANNED July test of the US antisatellite (ASAT) weapon against a live target in space has been delayed yet again, and is unlikely to be rescheduled before Congress renews a moratorium on such tests. The previous moratorium expired in March.

The official explanation for the delay is a technical problem with the instrumented target, designed to simulate the infrared radiation of a Soviet satellite and to monitor the ASAT's performance. The targets have been returned to their manufacturer, Avco Systems, for repairs.

According to congressional staff, however, problems with ASAT itself persist as well. Last December, the Air Force was seeking bids on a contract for the redesign of ASAT's manoeuvring propulsion package, an array of 57 miniature solid-fuel rockets that steers the 12-inch-long, non-explosive projectile into its target. One problem mentioned then was motor exhaust contaminating the infrared sensors that lock onto heat radiation from the target. The failure to resolve these problems is thought to have led the Air Force to delay the test scheduled for July; the ASAT has been returned to its manufacturers, LTV Corporation.

Under current law, the test against a target could proceed if the President certifies to Congress that the test is necessary to avert irrevocable damage to

national security, that it would not damage prospects for negotiating a treaty limiting ASATs, and that the administration is trying to negotiate such a treaty.

The House of Representatives last month passed an amendment to the 1986 defence bill that allows tests only if the Soviet Union tests first a dedicated ASAT system against a target in space. The Senate has adopted a weaker amendment, requiring only presidential certification of his efforts to negotiate a treaty. The US ASAT has so far been fired only at an imaginary target in space.

One suspicion all along has been that the delay in testing against a target, although originally the result of technical problems, has been dragged out for diplomatic reasons.

US officials made it known last week that Soviet negotiators in Geneva have informally proposed dropping their insistence on an end to Star Wars research as a precondition for any new arms-control agreement.

The Soviets are said to be willing to draw a distinction between laboratory research, which could continue, and development and field testing, which would be banned. The Soviet delegates were said to have singled out the US ASAT tests as one example of actions that would not be allowed. The Soviets are expected to submit this new position in a formal proposal shortly.

Stephen Budiansky

UK energy

Department puts money on wind

THE British government, which in 1975 spent £0.5 million on renewable energy research, has since taken a wait-and-see attitude towards unconventional sources of energy. Now, backed by reports from the Advisory Council on Research and Development for Fuel and Power (ACORD), it seems to be coming off the fence. Technologies that the Department of Energy (DOE) calls "winners" — wind, solar, biofuels and geothermal hot dry rocks — will be financed for further research and development, but "less promising" options, notably the £17 million research programme into wave power, will be halted until further notice.

Mr David Hunt, the DOE minister responsible for renewables, earlier this month defended both the amount of government spending and its choices. This year, £14 million has been earmarked for renewables research which, Mr Hunt pointed out, should be increased to £20 million from European and other sources. He claims that these figures demonstrate a commitment to renewables as part of the energy grid.

Questioned whether the increase of cash spending from £16 million in the five years 1975–80 to £68 million in 1980–85 may mask a decrease in commitment, Mr Hunt said that renewables technology "stands up for itself" and should not be judged in terms of the spending on other energy sources. In any case, he claims, the government's programme is "the most positive commitment by any [British] government" to renewable energy sources.

Backing the winners — and wind is "no doubt the most promising" — will mean that more money is channelled into the technologies that ACORD and DOE have labelled as "economically attractive" or "promising but uncertain", and which may make a significant contribution by 2025. Technologies judged less viable, such as converting wave energy into a more usable form, will not be funded but DOE will monitor developments abroad and be ready to change tack. Mr Hunt urged members of the renewable resources community to look beyond their hobbyhorses and to accept the commercial common sense of backing such sources as wind, in which Britain enjoys "world-beating" technology. Only then would these technologies be got "out of the research stations, off the drawing boards and into our daily lives".

ACORD's enthusiasm for wind reflects world optimism, especially in the United States, where wind turbine farms already generate a significant amount of energy. DOE plans to concentrate on large-scale machines, both horizontal and vertical axis, and emphasizes the export possibilities as well as the generation of energy for remote areas of Britain. Research ex-

penditures in 1984–85 of approximately £4.5 million were roughly double those of the previous year; £6 million is budgeted for 1985–86.

The significant British milestones are at Bugar Hill in Orkney and at Carmarthen Bay. At Orkney a 250kW horizontal axis machine, built as a prototype, has operated for 2,303 hours and generated 212,533 kWh; now a 60m diameter 3MW horizontal axis turbine has been approved for the same site. Work at Carmarthen Bay has centred on building a multi-megawatt vertical axis turbine. ACORD estimates for 2025 suggest a 1.6 million tonnes of coal equivalent (Mtce) a year contribution from such onshore sites. Offshore development is still at a theoretical stage, but plans for both national and international projects are afoot.

DOE is also backing passive solar energy, the direct solar heating of buildings, a potential winner that "makes good economic sense". The private sector, according to Hunt, will be encouraged to design buildings that "make the most of sun shine", thereby cutting heating and lighting costs by as much as 30 per cent. ACORD describes passive solar design as a technology that "has come to fruition and is ready to enter the market".

Unlike wind energy projects, in which many technical obstacles impede large-scale use, ACORD finds the impediment to solar energy to be customer diffidence. Potential customers must accept that a solar design does not entail putting up with an unattractive living arrangement. Passive solar's estimated contribution by 2025 is 2 Mtce per year for the domestic sector.

By ACORD's definition, biofuel is non-fossil combustible material, such as dry biomass (straw, wood), wet wastes (animal slurry, food industry wastes) and energy crops (trees or plants grown specifically for energy). ACORD sees organic wastes as the main biofuels in the next 20–30 years. Research is now to be concentrated on the best conversion technologies — direct combustion, thermal processing, anaerobic digestion — for the various forms of waste. ACORD predicts a 1.6 and 8 Mtce a year contribution from wet and dry wastes respectively.

DOE also plans to continue support for geothermal hot dry rocks because of promising experimental results, even though actual output is uncertain. Research is now centred at Rosemanowes, Cornwall, in a project run by the Camborne School of Mines. The idea is to cut holes to depths of 2,000–6,000 metres, artificially fracture the rock and heat water by circulating it through these reservoirs. The project is now waiting for results from a third hole that was recently dug; if these are positive, more and deeper holes will be drilled.

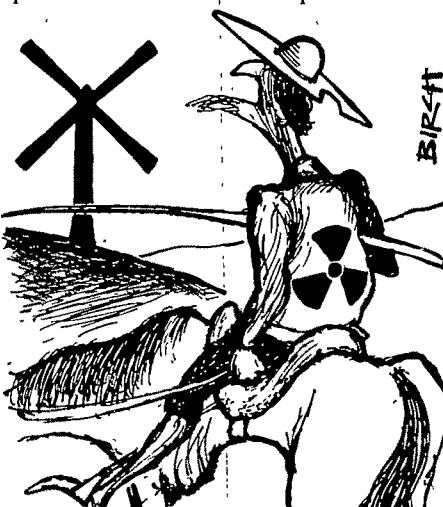
Achieving technical feasibility, however, is not the final hurdle before a renewable energy project is built. These projects often have high start-up costs that are less easily dissociated from the project than are the clean-up costs of conventional energy sources. According to Mr Hunt, allowances will be made for alternative energy sources in Britain. **Elizabeth Collins**

Wind power

West Germany backs out

Hamburg

GROWIAN, (for *Grosse Windenergie Anlage*) the largest wind-driven power plant in the world, is to be dismantled. Situated on the estuary of the Elbe, it could, at least on windy days (around 300 a year), produce 3 MW of electrical power.



Developed during the oil crisis, the windmill was constructed in the early 1980s at a total cost of DM90 million (£22.5 million). Now cracks have appeared in the rotor framework which would require a further DM10 million to repair. Dr Heinz Riesenhuber (CDU), Minister for Research and Technology in the West German government, has decided instead to close the gigantic propeller down next year and to have it dismantled later. The call for the development of alternative sources of energy has become less strident now that the costs of conventional sources have stabilized, which is why the wind energy programme begun by the Social Democrat/Liberal coalition faces its end.

So far this programme has cost DM200 million, a small sum compared with the DM1,600 million invested in nuclear energy, mainly in the fast-breeder reactor plant at Kalkar. The GROWIAN project was at the outset criticized for its enormous size, and for the expected difficulty of operating such a large machine. Its cancellation is seen as a success for the nuclear lobby, which has always maintained that alternative sources of energy have no future in West Germany. **Jürgen Neffe**

French biotechnology

Making high-tech champagne

BIOTECHNOLOGY is about to be applied to those distinctively French wines and cheeses. That is the novel component of the recently approved biotechnology programme of the ministry of research and technology.

The French government and the agro-food manufacturers, France's second largest industrial community, are worried that their traditional methods are too unreliable, variable and sensitive to uncontrollable factors such as the weather to secure their place in the world markets against high-technology producers in California, South Africa and Australia. So the French food producers have decided they will use science to help.

Champagne will be one beneficiary. One of the most difficult elements of champagne technology, *rémuage*, is the removal of spent yeast from the wine after the second fermentation that produces the "fizz" and the flavour. This is already being changed by the use of "fixed", pelleted yeast. Moët-Hennessy, the producer of Moët et Chandon and many other well-known champagnes, is carefully introducing the new methods.

The company says there is no detectable effect on the flavour, and that the advantage will be that *rémuage* will take only three days, compared with the present laborious three months of slowly turning and raising the bottles until they are cork-down, followed by a swift opening and closing of the bottle by an expert to blow out the unwanted yeast. With fixed yeast, the same blowing-out is necessary, but the yeast pellets sink far more rapidly than individual yeast cells.

The saving of storage time will increase by 80 per cent the capacity of the champagne cellars that burrow throughout the champagne district, says Moët-Hennessy, which would help iron out the immense fluctuations in champagne production caused by the weather. (Champagne, one of the world's most northerly wine-growing areas, produced 300 million bottles in 1983, but only 80 million in 1981.)

The vines themselves are also under study. The small Programme Nationale de la Vigne, established three years ago by the research ministry, held its first scientific conference last spring and raised hopes that gene cloning and transfer techniques together with cell fusion and tissue culture might well produce useful new varieties. In the past, it has taken between 20 years and a century to make a new clone adapted to a particular soil and climate; this time must be shortened if new varieties resistant to disease or less sensitive to spring frosts, for example, are to be developed.

Cheese production, usually by small local makers, is also in question in a dairy industry which was shocked to hear re-

cently that Japan, which is on a wave of fashion for French cuisine, has begun to manufacture its own high-tech but tasty Camembert. This apparently uses French organisms but Australian milk, and thus represents a large market which the small French producers have almost totally lost. Scientifically, the questions again are how to achieve constancy, quality and volume.

At the ministry, the view is that after three years of government aid, biotechnology is already well established in the large French drugs, vaccines, bioreactants, fine chemicals and medical manufacturers, but that the small agrobusinesses now need a helping hand. Many of the companies are too small to have any research effort at all. Hence, according to Pierre Douzou, director of the new gov-

ernment *programme mobilisateur* for biotechnology, seven out of ten of the industrial advisers for the programme will be drawn from the agrofood industries, and the emphasis of the new programme, supported by FF 115 million (£11 million) of seed money for 1986, will be in agriculture and food.

The new trend has also been emphasized by Douzou's further appointment as chairman of the scientific council of INRA, the national agricultural research organization, which has "many fine scientists" but has been historically somewhat isolated from other French scientific communities, particularly those supported by the national and medical research councils, CNRS and INSERM, and even from the universities.

Douzou sees his job as helping INRA director Jacques Poly to "opening up" INRA to the rest of science.

Robert Walgate

European professions

Plan to make diplomas travel

Brussels

THE European Commission has drafted legislation laying down the principle of mutual recognition of all higher education diplomas throughout the Community. The aim is to facilitate the mobility of those exercising a profession. The European Commission seems to think this can be achieved without harmonization of national higher education systems. But the draft directive is very much at the beginning of a consultative process in which the European Parliament, industry, employers and various lobby groups, as well as governments, will want their say.

The measure aims at tying up a rather large loose end in Community law, whereby it is illegal to discriminate on the grounds of nationality, so that Community citizens have the right to set up their professional domicile in any of the ten countries of the European Economic Community (EEC), but where paradoxically only nationally acquired qualifications are recognized in order to be able to exercise one's profession. The only exceptions are doctors, veterinarians, dentists and architects, for whom mutual recognition of diplomas already exists.

But whereas in these cases academic curricula were to some extent harmonized throughout the Community as well, this time the Edonnino committee, mandated by heads of state at Fontainebleau in June 1984, recommended, instead, just an overall system of mutual recognition and trust, rather than the time-consuming harmonization of training courses.

The draft directive covers all professions requiring three years of higher (university-type) education which have followed a higher secondary course — *baccalaureat*, *abitur* or A-level.

It thus applies equally to paramedics

(physiotherapists, opticians, psychologists), the scientific community (engineers, geologists, agronomists), lawyers, journalists, economists, statisticians and teachers.

Information on professional recognition would be available from a computerized network linking up centres that already exist.

While the proposal is clearly an expression of political will on the part of governments towards the European ideal of complete mobility of citizens, making it easier for those whose home market is saturated to move to another EEC country, in practice it may come up against the protectionism and corporatism that characterized some of those professions.

Secondly, it is clear, as the proposal points out, that despite some measure of similarity or comparability, differences between the education and training systems in a given profession will still require the newcomer to obtain further experience or training in the proposed country of establishment. The proposal allows for a maximum period of three years but makes no budgetary provision at Community level, nor does it specify who will pay. Under Community law, a directive lays down the aims but leaves the means of achieving them to the member states. This could give rise to different financial constraints in different member states.

Achieving some level of uniformity between national education systems could further enhance the mobility aimed at by the draft legislation, but there is no provision for harmonizing education in the Rome treaties. Even if EEC education ministers do meet to discuss equal opportunities for students, this is an area in which they jealously guard their sovereignty.

Anna Lubinska

Polish universities

Doubts about concessions

POLISH universities seen at last to have won concessions from the government in their campaign against the proposed reform of the 1982 Higher Education Act. According to the leading Warsaw weekly, *Polityka*, the government Committee for Social and Political Affairs, in charge of drafting the reform, has given way on five major points. But many academics fear that these concessions are essentially cosmetic, designed to allay further, last-minute criticism of the amendments which, it is believed, will go before the Sejm (Parliament) in the near future.

According to *Polityka*, the Committee for Social and Political Affairs has decided:

- to drop proposals to replace tenure for senior lecturers and professors and to replace it by a contract system;
- to drop the proposal that rectors, pro-rectors and deans should be appointed by the Minister of Higher Education and Science instead of being elected by the universities themselves;
- to retain elections to the Main Council for Higher Education, the 70-person body representing all Polish universities and higher education schools (except the private Catholic University of Lublin);
- to allow universities to decide their own statutes instead of having to conform to a standard form of statutes dictated by the Minister;
- to retain some form of student "self-government committees", instead of replacing them by bodies linked to party youth organizations.

Although, formally, there was a major public debate on the proposed changes earlier this year, the *Polityka* article is the first open admission that the universities (as represented by the Main Council) are diametrically opposed to the government over the changes.

Moreover, *Polityka* cited several leading academics who contradict the assertion of the Minister of Science and Higher Education, Dr Benson Miskiewicz, that falling academic performance over the past three years is clear proof that the 1982 act has been used to "destabilize" the universities. The rectors of Warsaw Polytechnic University and the Jagellonian University of Kraków, in particular, are quoted as saying that while, unfortunately, there has been a decline both in the percentage of students completing their courses and in the quality of their final dissertations, there is considerable doubt as to whether this is a result of the 1982 act. This apparent "openness" of the *Polityka* article has given some encouragement to the more optimistic academics.

On the other hand, the less optimistic point out, there was never any real likelihood that tenure would be abolished.

Even a tenured lecturer can be dismissed by special decision of the Council of State (as happened recently in the case of Dr Lessek Nowak, a social scientist at the University of Poznań) and the proposal was probably included in the draft as a bargaining counter. The election of university officials will be carried out by a senate which will consist almost entirely of senior tenured staff, and without the participation of representatives of the student body and non-teaching academic employees. The abolition of elections to the Main Council is said to have been proposed, as a result of the vehement opposition shown by that body to the changes, as a means of reducing its power. Although it will not be changed into a puppet body of ministerial appointees, the Main Council still loses its decision-making powers, so that, under the reform, the minister will have only to "consult" the Main Council on terms of academic employment.

Even more serious, the minister will still retain what many see as the greatest threat of the reform, the right to dismiss or suspend any university official or lecturer, expel or suspend any student, and close down or suspend any department, without appeal or independent disciplinary hearing, if he deems it "in the public interest".

Furthermore, one should read not only *Polityka*, which still enjoys "by inertia" something of its pre-1981 reputation for a liberal outlook (by Polish standards) but also the Party daily *Trybuna Ludu*, which, only a few days previously, had given its own views of the reform. According to *Trybuna Ludu*, opposition to the reform is virtually equivalent to opposition to the Party, and, it implied, under the amended act, university employment would be dependent on an oath of loyalty to the concept of a socialist university. The idea of such an "oath" came as a surprise to most academics, who throughout the whole campaign have been largely dependent on "leaks" to the Solidarity underground press for information about what the government was planning. **Vera Rich**

British geology

Crying in the wilderness

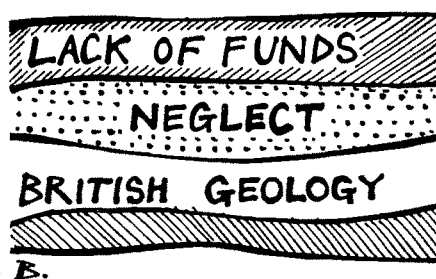
THE saga of the declining budget for British geology took a new turn last week. In the most public and formal *cri de coeur* from the geological community so far, nine presidents and chairmen of major professional bodies* published a tract in which they summarize the present economic circumstances of their disciplines and the dire consequences they anticipate if fundamental changes, including an increased proportion of mineral taxes to be

84 amounted to 0.0043 per cent of the latter figure.

The signatories of the tract go on to outline the "impasse" with which they are confronted. In answer to the question of whether academic and institutional research is worth saving, they say, "the common government response is that if industry wants it, industry will pay for it. Industry usually replies that, as they have already paid for it in taxes and royalties, government should pay." The government's way out, say the signatories, is to tell the universities to fund research from consultancy service, an approach which, while being increasingly adopted, is bringing universities into conflict with the private sector.

The solution proposed is, in the short term, an injection of £15 million "earmarked specifically for the geological sciences... adequately to fund systematic surveys, to computerize national data-banks, to re-equip research centres of excellence and to allow UK participation in major international projects". And NERC? The document uses weasel words: "The role of NERC needs to be evaluated and the rationality of its Corporate Plan assessed" **Philip Campbell**

* Signatories: P. Allen (chairman, British National Committee for Geology), C. H. Holland (president, Geological Society), D. C. Ion (president, Institution of Geologists), W. G. Yuill (president, Institution of Mining and Mineralogy), J. Birks (president, Institute of Petroleum), P. I. Allsop (president, Institution of Mining Engineers), M. G. Audley-Charles (chairman, Committee of Heads of University Geology Departments), R. F. P. Hardman (chairman, Petroleum Exploration Society of Great Britain) and J. R. Vail (chairman, Committee of Heads of Polytechnic Geology Departments)



spent on research, are not soon made.

The decline in research commissioned by government departments, as well as cuts in funds from the Natural Environment Research Council (NERC) has led to a virtual halt in strategic surveying by the British Geological Survey (BGS) (see *Nature* 311, 499; 1984). The new document, sent to Secretary of State for Education and Science Sir Keith Joseph last week, summarizes this position but puts it in the most general economic terms. In 1983, the total mineral production (including oil) by Britain was worth £24,000 million; the tax and royalty payments from North Sea oil alone in 1984 amounted to £9,000 million. Funds from NERC for the geological sciences in 1983—

Nuclear insurance

US Congress prepares new rules

Washington

AN obscure decennial ritual that will determine who pays in the case of a nuclear accident is about to begin again. The Price-Anderson Act, which sets a limit on the financial liability of nuclear utilities, expires next year; lobbying by industry and its opponents is already shifting into high gear.

The act, first passed in 1957, was designed to remove a major obstacle to the adoption of nuclear power by electric utilities: the risk of a crippling financial burden in the event of a catastrophic nuclear accident. The act removes the right of injured parties to sue anyone but the utility, and places a limit on the liability of utilities. (The figure depends on the number of licensed reactors in the country; it is currently \$630 million.)

In its original form the act provided for the federal government to play the role of chief insurer. That provision was altered when the bill came up for renewal in 1967 and 1977 and each utility is now required to carry \$160 million in insurance; in addition, if an accident occurs that causes damage in excess of that amount, each reactor will be assessed up to \$5 million—a sort of after-the-fact insurance premium. The federal government is now liable only for accidents at its own facilities, and only up to \$500 million.

Industry, in pressing for a renewal of the act, argues that it is not a subsidy to the nuclear power industry, but rather a form of "no-fault" insurance, akin to worker's compensation. Without the liability limit, industry argues, victims of an accident might receive even less—judgements of more than a few hundred million dollars would bankrupt the average utility.

Critics point out, simply, that a major accident could easily cause damage to the extent of tens of thousands of millions of dollars; they claim that by shielding the industry from this risk, the act skews economic incentives to increase safety.

Opposition is also emerging from states that are candidates to receive federally operated depositories for high-level reactor waste, as the act limits the amount that victims of an accident at such a facility can recover to \$500 million.

Even if Congress fails to renew the act, however, the reactors already licensed would probably continue to enjoy the protection of the act. US courts have long held that legislation such as the Price-Anderson Act represents a financial contract that cannot be undone by subsequent legislation. At issue is whether new reactors coming on line after 1 August 1987 will be covered.

Several bills have already been filed in Congress:

●Simpson-McClure (S 1225) would maintain the basic framework of the act while

increasing the liability limit to \$2,000 million.

●Morrison (HR 2524) would provide for full compensation for any accident involving high-level waste.

●Hart (S 445) would repeal the liability limit while retaining the current insurance scheme; victims would be free to sue any liable party if damages exceed \$630 million.

Another alternative, endorsed by the Nuclear Regulatory Commission, would provide full compensation to victims by assessing after-the-fact premiums from all reactors at \$5 million a year for as long as it takes.

Stephen Budiansky

Genetic engineering

Rifkin set back

Washington

THE US District Court of the District of Columbia has denied the latest attempt by anti-genetic-engineering activist Jeremy Rifkin to put legal obstacles in the way of a deliberate release experiment. Judge Aubrey Robinson ruled that the National Institutes of Health (NIH) could not, as requested by Rifkin, be compelled to regulate a proposed field test of a genetically modified bacterium intended to protect crops against frost damage. The experiment, proposed by Advanced Genetic Sciences Inc., has now been submitted instead to the Environmental Protection Agency (EPA).

The experiment was recommended for approval in June 1984 by NIH's Recombinant DNA Advisory Committee (RAC), but the director of NIH withheld his approval to avoid applying different standards to private companies, as opposed to academic researchers: a previous injunction forbade NIH to approve an almost identical experiment by Stephen Lindow of the University of California until NIH prepared an environmental impact statement.

Dismayed by the legal circus surrounding the Lindow proposal and the prospect of long delays, Advanced Genetic Sciences withdrew its application (which was in any case only voluntary) and went to EPA, which has claimed legal authority over deliberate releases of some recombinant organisms.

Rifkin petitioned the court to rule RAC's original recommendation of the Advanced Genetic Sciences experiment invalid, on the basis of obscure legal technicalities; he then sought to compel NIH to forbid it.

In dismissing both petitions, Judge Robinson made plain his puzzlement with Rifkin's argument, and the experiment now lies firmly in the lap of EPA.

Tim Beardsley

Leprosy vaccine

Malawi trial to be launched

ALTHOUGH more money has yet to be found, the British Leprosy Relief Association (LEPRA) has announced the launch of a full-scale trial of a newly developed leprosy vaccine in northern Malawi in 1986 in conjunction with the government of Malawi, the London School of Hygiene and Tropical Medicine (LSHTM) and the World Health Organization (WHO).

About 15 million people suffer from leprosy, and although drug treatment is available, it can be very slow and often too late to prevent irreversible neural damage. Excitement is understandable at the development of a potential vaccine, which has been made possible largely by the collaborative efforts of a group of scientists within the WHO Special Programme for Research and Training in Tropical Diseases (TDR) coordinated by Dr Barry Bloom of Albert Einstein College of Medicine, New York.

The vaccine consists of heat-killed *Mycobacterium leprae*, the causative agent of the disease, purified from the tissues of the nine-banded armadillo, the best animal host for growing large numbers of bacteria which cannot be grown *in vitro*. Four colonies of armadillos are maintained by TDR for this purpose. Safety trials have been completed in Norway, where leprosy was eradicated as recently as 1950, and a large-scale non-random trial of 60,000 leprosy contacts is under way in Venezuela which will be completed later this year.

The Karonga district of Malawi has been chosen for the first population-wide trial largely because prevalence of the disease is rather high (1–2 per cent) and because of the groundwork laid by LEPRA's comprehensive evaluation programme in this region directed by Dr J.M. Panninghaus and coordinated by Dr P. Fine of LSHTM. Over the past six years, at a cost of approximately \$500,000, all 120,000 residents of the region have been examined and interviewed, and improved serological tests have been developed. About 1,700 leprosy cases are present and up to 240 new ones have appeared each year.

There is evidence from earlier trials that vaccination with Bacille Calmette-Guérin (BCG) against tuberculosis can provide some protection against leprosy and, indeed, animal experiments and therapeutic work in Venezuela suggest a vaccine containing both killed *M. leprae* and BCG is most promising. The aim therefore is essentially to compare this mixed vaccine with BCG alone in a randomized control trial of the whole population except those who do not wish to be involved and those who will be excluded on the grounds of age, ill-health or already diagnosed as leprosy cases.

Nigel Williams

Creationism down under

SIR—Tony Thulborn is to be congratulated on his exposure of the Creation Science Foundation in Queensland (*Nature* 9 May, p.89) and will be pleased to know that the Australian APE (Australian Association for the Protection of Evolution) is not entirely alone.

The Institute of Biology in Australia (IBA), which is a branch of the Institute of Biology and on the road to total independence, has already challenged creationists in a major symposium, "In defence of science — A response to creationism", held in Sydney with an audience of more than 200 people on 9 March. The meeting was sponsored by IBA jointly with the Linnean Society of New South Wales, the Royal Zoological Society of New South Wales and the Australian Museum Society. Speakers successfully countered persistent (and ill-informed) questioning by creationists, some of whom freely admitted to teaching creationism unofficially in science classes at all levels, in schools in New South Wales, thus confirming Thulborn's suspicions.

The proceedings were videotaped by staff from the Commonwealth (Australian) Department of Education. A short film will be released to schools soon. The papers and discussion (edited by Dr Frank Burrows) will be published by the University of New South Wales as the first volume of a new series entitled *Australian Studies in Biology*.

The fight against creationism will be continued at the second annual general meeting of the institute in Brisbane on 5–7 July. A motion will be put affirming *inter alia* that creationism is not a science and thus has no place in any biology syllabus in Australian schools.

JOHN SKIDMORE

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SIR—Michael Cavanaugh's implications in his article "Scientific creationism and rationality" (*Nature* 16 May, p.185) cast some doubts as to whether creationism can muster sufficient support from *bona fide* academics and especially from PhDs holding established positions in reputable scientific organizations. One of the main reasons for this state of affairs, of course, is that creationists are threatened by their own colleagues and by legal action by their own school boards and institutions if they do not keep a strictly low profile either publicly or in their own class rooms. Fear of loss of respect and prestige, not to mention endangering their own jobs, is what keeps highly qualified creationists in their ivory towers (even to the extent where they avoid open association with outspoken creationists). I speak from person-

al experience and from the knowledge of what other creationists are going through.

C. K. PALLAGHY

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SIR—Having reread *Genesis* for the first time in several years, prompted by Cavanaugh (*Nature* 315,185; 1985) on creationism, I still fail to see the difference between creationism and evolutionism apart from the time-scale, which could easily be resolved by postulating that one theological day corresponds to a large number of millennia, in which case we are still waiting for the events of the eighth day. If we accept this approximation, then the only difference between evolutionism and creationism is the question of the catalyst.

As the hypothesis of the creationists is a supernatural force whose existence can be established only in the after-life, it would seem reasonable to withhold judgement until after death when the hypothesis will be confirmed or refuted. Given the many millennia which modern evolutionists would consider to correspond to a single creationist's day and the relatively short time since the creation of man, which marked the end of the sixth day and which was followed by a day of rest, it would seem likely that death for most of us alive today will be an earlier event than the creationist's eighth day.

Further speculation is futile, providing creationists will concede to the evolutionists that the theological day is indeed in excess of 24 worldly hours.

R. T. D. OLIVER

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Jobs in Spain

SIR—Recently, because of the large number of technical and academic professionals emigrating during the Franco years, King Juan Carlos let it be known that no Spaniard should ever again feel that he (or she) could not work in Spain. Unfortunately, the inertia of 40 years of scorning knowledge and its human heritage leaves the royal wish unheeded in the spheres which could do something about it.

During the Franco era and even afterwards, a small but steady stream of young Spaniards left Spain to do research or to benefit from higher education abroad. Many now wish to return. The national propaganda (words and wishes) wants them back; the system, however, is not willing. In Spain, positions at universities or research institutes are awarded by peculiar and primitive ritualized examinations called "oposiciones". In principle (and on paper) they are open to any Spa-

niard. The reality is different; only Spaniards with a Spanish degree can apply. In short, a degree of any kind from a foreign university (regardless of its name or place) is not valid and the knowledge that accompanies it is questioned. Of course, the bureaucracy provides a simple way out: "convalidacion" (validation). One can apply to validate one's degree; what this involves varies and, in general, is inversely proportional to how many influential personalities the applicant knows.

It is a sad and deplorable situation, particularly in the euphoria of Spanish integration in Europe. The absurdity of the situation is clear from the fact that any Spaniard with a Spanish PhD can obtain a job in Europe or the United States, without any bureaucracy or any more questions than those related to the job; any Spaniard with a PhD, from, say a US, English or German university has to fall into a "windmill battle" with Spanish academic authorities and thus is not eligible for a job in his/her own country.

It is important that these ancestral prejudices disappear. They are probably a consequence of the isolationism that has characterized the Iberian peninsula for centuries. The accession of Spain to the European Community attempts to break the barriers that extend above the Pyrenees. Let us hope that it breaks all barriers. ALFONSO MARTINEZ-ARIAS
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Scientist arrested

SIR — We have heard from scientists in Poland that their colleague, Zbigniew Gluza, has been arrested. Gluza, aged 33, was employed at the editorial board of the journal *Informatics*—the leading publication in the field in Poland—and is a member of the Polish Informatical Society. He was also a member of Solidarity.

Zbigniew Gluza has become a victim of the regime and an example of the planned programme of repression being suffered by Polish intellectuals.

During a random inspection in the street in Warsaw, the Polish *milicja* found some popular underground literature in Gluza's car. It should be pointed out that these underground bulletins play an important role in Poland, because official publications do not carry a genuine news digest—only officially edited reports which are usually distorted by ideology and its powerful instrument, censorship.

Letters of protest are being written to Polish embassies, consulates, cultural and scientific institutions. It is to be hoped that international groups and societies will add their weight to the protest. The efforts of private individuals will also be welcomed.

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DEPARTMENT OF SCIENTIFIC AND INDUSTRIAL RESEARCH

Science at a very great distance

Australia and New Zealand are in a different part of the world from most other places, as the cover shows. They are also a long way from each other, geographically and in other ways. This survey of science in the two countries is necessarily far from complete. It is offered as evidence of the vitality and promise of what is happening in these interesting places.

Australia's technical revolution

THE government of Australia is in danger of talking itself into a muddle about science and technology. Australia is blessed not only with great natural resources and a splendid climate but with a superb scientific enterprise. The government has rightly diagnosed the economic decline of the past few years as the consequence of Australia's failure to increase exports of manufactured goods to compensate for the diminished value of exports of primary materials, agricultural produce and minerals, itself a consequence both of the world-wide recession and, in agriculture, of protectionism (as by the European Communities). For this reason but also by the inspiration of Mr Barry Jones, the Minister for Science, Mr Bob Hawke's Labor government has been, during its two years in office, embarked on a great drive to turn Australia into a technological community. It is a brave and laudable ambition, which should have enlivened the technical community. Why, then, are the people who work in academic and research laboratories more anxious, even despondent, than they have been for many years?

A large part of the trouble is that the technical community, while sharing the government's aspirations that technology should be made economically more valuable, fears that the government will not put its money where its mouth is and that its enthusiasm will get the better of its judgement. Last year's failure in the budget process to make a substantial increase in the funds of the meagrely supported research grants scheme for academics is worrying, as is the requirement that the public research organization (CSIRO) should do more (industrial innovation, especially) with less. And there is little sign that the Hawke government's conviction that the supply of skilled people from Australian universities must be increased will be followed by commensurate support. Will the government show that it means what it says in next month's budget?

Exorcising anxiety of the second kind will be more difficult. The prophet of the revolution the government hopes to work, Mr Barry Jones, is politically more radical than pragmatic Hawke, but he properly

appreciates that basic science is as crucial a part of technological change as is the engineering of innovation. The practitioners of the revolution are harder men who share Jones's vision and impatience but not his understanding. Collectively, they give the impression that scientists who are not working for industry are a bunch of layabouts. The obvious danger is that they will intemperately enforce changes that damage Australian science without yielding much in the way of economic benefit. Aesop's fable about the dog, and the reflection of his bone in the water, applies.

The torrent of public introspection since 1983 about the engineering of Australia's revolution has been more confusing than clarifying. This month's second draft of the "National Technology Plan" (from which Mr Jones mildly dissents) is even fuller than last year's version of specific goals, and actions to be taken by government and others. Even in a country as used to being overgoverned as Australia, the proposals seem over-dirigiste; is it possible, let alone desirable, that part of the government's subsidy of universities should be "earmarked" for "mission-oriented basic and applied research" intended to make existing industries more competitive and to create entirely novel industries? That is what the plan says.

A more serious weakness of the public argument about the future is that it seems innocent of serious consideration of what Australia might be like economically if and when the revolution has come to pass. Australia seems ready to turn its back on the traditional sources of its wealth. In the dash for a new prosperity, and with the knowledge of what the foreign exchanges have been saying this past year about the dangers of over-spending, the government's motto seems to be: "If people don't want our beef, we'll do research on something else". And so, inevitably, there is a new strategy for research, with priority objectives clearly spelled out: biotechnology, information, robotics and the application of computer-aided design to manufacturing technology and materials. Australia, with its 15 million people, is bursting to follow every European country in attempting to simulate what has been happening in the United States and

Kiwi alert

NEW Zealand's scientists face hard times. The government has decided progressively to lower the budgets of the major research organizations over the next three fiscal years. There will be further cuts in real funds, particularly in the universities, as inflation, now running at 15 per cent, makes itself felt. For the government, the falling budgets are regarded not so much as "cuts" as incentives for the recovery of funds from elsewhere. The gap that opens up as grants are reduced is meant to be filled by increasing the amount of money taken in from industry and from other government departments by contracts and by the sale of services. In a nutshell, "the user must pay for science".

In as far as they are to suffer financial austerities, scientists find themselves in much the same boat as many others in New Zealand. Despite its celebrated anti-nuclear stand, the new Labour government has brought in tough monetarist policies. It inherited a massive deficit from the outgoing National Party government and decided that a sharp shock was needed to wake New Zealand to the harsh economic realities. The first action of the new Prime Minister, David Lange, after winning the election last year was to devalue the dollar by twenty per cent; subsequently, it was allowed to float and its value has fallen further. Import licensing systems which had long protected local manufacturers were done away with, along with controls on financial markets. Some farm subsidies were also thrown out. Next, there is to be radical overhaul of the tax system aimed at slashing personal taxes and passing the bill to goods and services.

It is not hard to see why Lange decided on drastic action. In the mid-1950s, the country was the third richest in the world measured by per capita income. Now it is close to twentieth. The cause of the decline can be summed up in a sentence: the balance between primary and secondary industries is not appropriate to keep a small country in prosperity. In New Zealand, primary industry means agricultural products, principally meat, wool and dairy, making up 80 per cent of its exports. Markets for them are becoming harder to find and more and more unstable.

The solution to the problem is, in one

AUSTRALIA and New Zealand, until recently among the most prosperous countries in the world, have fallen on hard times. In each case, the explanation is that world demand for primary products has fallen. Each country has responded by setting its sights on the exploitation of science and technology to make products that will find new markets. For what it is worth, both countries have also within the past two years elected governments from the left of their political spectra, Mr David Lange's Labour government in New Zealand and Mr Bob Hawke's Labor government in Australia. (The word "programme" is also spelt with a single "m" in Australia.)

This survey of science in both countries is necessarily incomplete. *Nature* wishes to thank the many scientists and politicians in both countries for their helpful courtesy and also Dr Jeffrey Sellar, *Nature's* Canberra correspondent, and his predecessor, Dr Vimala Sarma.

NOTE: \$A1 = £0.51; \$NZ1 = £0.35

Japan Nobody seems to have thought much about the possibility that when demand for the traditional products is depressed may be the worst time to rob them of research support.

Yet what is wrong with Australian industry is not that Australians are unenterprising, or that Australian scientists are unimaginative or even that Australian workers are lazy — these are the opposites of the truth — but that Australia created for itself in the comfortable 1960s and 1970s an unreal economic framework whose abandonment not even the most radical politicians can advocate (in public). Australia has indulged xenophobic instincts about capital investment (wholly-owned subsidiaries of overseas companies are bad) and chauvinistic instincts about overseas trade (high-cost local manufactures behind tariff barriers are preferable to cheap imports). The result is that Australian manufacturing industry is inward-looking, persuaded that its only worthwhile market is Australia. So it is no surprise that Australian imports of technologically-based products are worth eight times the corresponding exports, or that the volume of research carried out by Australian companies is tiny (For Australian companies, the domestic market is not big enough to support full-blooded research; overseas companies do their research at home, although ICI and Kodak are exceptions.) Yet Australia's determination to become a technological economy has been prompted by the recent discovery of this unsurprising state of affairs.

This is why Australia could best satisfy its ambition to become a technological community by liberalizing the framework which has so far held it back. Mr Hawke and his Treasurer, Mr Paul Keating, have spent the past few weeks battling for a radical reform of domestic taxation

(which is a good cause, but one in which they will only partially succeed). To make their technical revolution happen, they must also reform the external economic relations of Australia. Devices such as the 150 per cent tax allowance (see p.189) will no doubt help, but can be no more an assurance of success than is the palliation of symptoms a cure for a disease.

What the government must appreciate is that, with 15 million people, it cannot be (and does not need to be) a powerful source of innovation and production across the whole field which it now wishes to explore. Indeed, Australia would be on safer ground if it were to build more energetically on the strengths it has already. The application of biotechnology in agriculture (animals as well as plants) is full of a promise that has been recognized. The reasons why Australia ships metal ores and the coal with which to reduce them to countries overseas (Japan particularly) is a puzzle, but one in economics Australia's apparently perverse determination not to add economic value to the uranium it sells abroad is political in origin, but a costly self-encumbrance. Australia's medicine has and deserves the highest reputation internationally, where are the pharmaceutical industries that would have sprung up elsewhere?

The risk in the indiscriminating course the government seems bent on following is that the quality of the present scientific enterprise will be undermined. That is what alarms many academic scientists. A modern state such as Australia needs to be in touch with what is happening in the whole field of science (which is why there should be no shame that many CSIRO laboratories are able to hold a watching brief on what may be happening elsewhere). There is no harm in offering people incentives to see their bright ideas turned into saleable products, although it is absurd that there should be such pressure from the government to ensure that Australian innovations are exploited only by Australian companies. But to expect that every working scientist should contribute directly to the revolution is a recipe for disaster.

The government's greatest need is for patience. It is right to look for a technological revolution in Australia, but not to expect this to happen overnight. It does not much help that it has chosen this time to review the operation of pretty well every important organization spending money on science. Might not some of them be allowed to get on with what they do already? (CSIRO, the largest public research organization, is in a different case; its management structure is not appropriate for the functions now expected of it; see p.189). Although part of the trouble is the short interval between elections (never more than three years), the government must learn to recognize the excellence of the science enterprise it has inherited and learn to meddle less. □

sense, obvious. First, value must be added to New Zealand exports: more pulp and paper and less timber, more carpets and less wool. Second, the range of products must be increased; new types of cheese must be developed, new fruits grown, and meat processed in new ways for new markets. And third, more manufactured goods must be developed in areas where New Zealand is "believable"; farm equipment, software packages for farm management, portable communications equipment and the like.

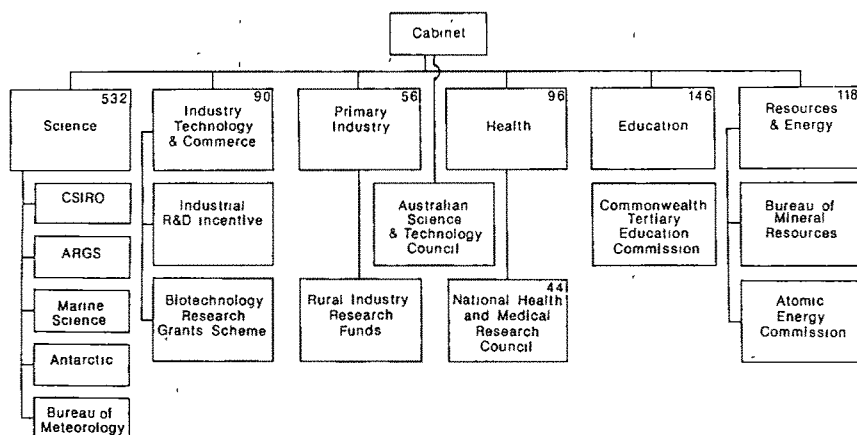
How to bring about these changes is less than obvious. Lange's policies have so far proved a major shock to the economy. Growth has fallen to less than one per cent this year compared with four per cent last year. But in a phrase that will be familiar at least in Britain, Lange says that New Zealand cannot continue to "live beyond its means".

The bludgeon that has been applied to the economy is now to be applied to science. The government may yet regret what it is doing. New Zealand's problem in research is that there is a great deal of talent, of which a large part lacks the means to show what it is capable of.

The imaginative technological changes needed in the economy require that talent be put to use. But simply trying to harness science to industry through contract work is unlikely to be productive, for industry does not have a clear enough idea of its own long-term interests to be able to direct research sensibly. Nor does it understand that without basic research before long there will be no applied research worthy of the name. The government pays lip service to the idea that science must be promoted. It seems not to see the danger in reducing budgets indiscriminately.

There may be an explanation. All the signs are that the government is remarkably ignorant of science and technology and generally holds scientists, as against managers, in low regard. Occasionally one hears that the "Japanese way" should be followed; and that all science should be put in the service of industry. But that is not the real situation in Japan. If anything distinguishes that country, it is the premium placed on being well-informed about scientific matters: the Prime Minister heads the key scientific policy-making body and there are reams of reports and statistics. In contrast, it was only a few months ago that anyone in New Zealand had more than a half-accurate idea of how much industry was spending on research. What the government should be doing is finding out what the nation's scientists — and the universities constitute a major pool of under-used expertise — can contribute. And that identifies what seems to be a major weakness of the present administrative system. No powerful voice represents science to a high level of government. But without giving science high priority, how can the technological change on which New Zealand has set its mark ever be achieved? □

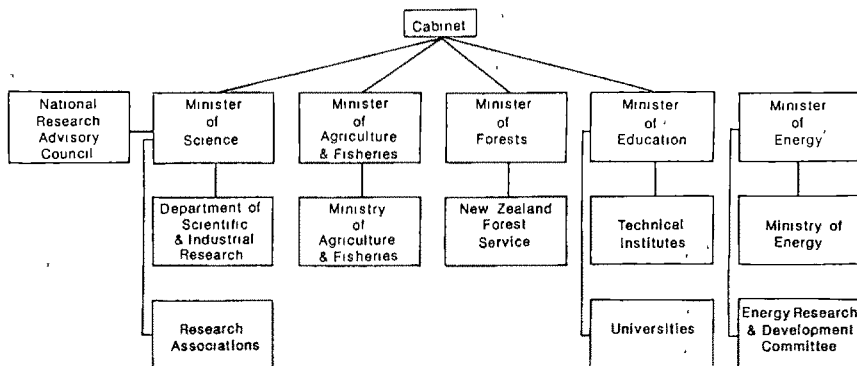
How science is run in Australia...



Most government ministries spend some money on research. (Defence is excluded from the diagram above.) The numbers attached to each ministry are the estimated expenditures in 1984-85 on research and development in millions of Australian dollars. The particular complication of Au-

stralia is that the federal structure shown above is paralleled by a comparable pattern of administration at the state level; especially in agriculture, states are substantial spenders on their own account (while research funds are also recruited by means of a levy on certain products).

... and New Zealand



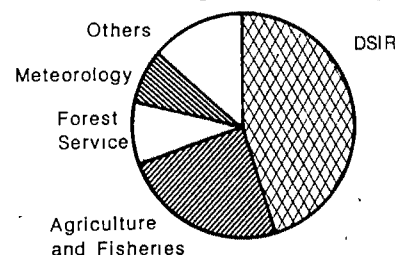
As well as those shown above, two other ministries have research arms: Transport (New Zealand Meteorological Service), and Works and Development (Water and Soil Research and Survey group). Grants

are also provided by the Medical Research Council to universities, hospitals and its own small research units for dental research, virus toxicology, immunopathy and alcohol research.

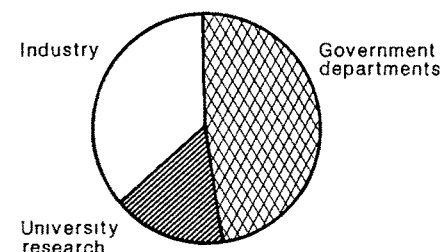
Science and technology



FUNDING for New Zealand's research and development for the year 1984-85. Government spending is \$NZ150 million, the universities \$NZ200 million and industry \$NZ69 million. Had the chart been drawn a little earlier it would have been divided into quite different slices, for it has only just been discovered that industry spends a considerable sum on research. There is a simple reason for that: because expenditures on research for new products (as against current product lines) are not tax-deductible, research costs have consistently been hidden under "maintenance costs" and other such categories. A new survey has revealed the above figures and in doing so destroyed a recent OECD analysis which centred on the belief that industrial research and developed effort was tiny.



THE Department of Scientific and Industrial Research (DSIR) takes the major share of the government funds.



Texture of diversity in Australian science

THAT Australian science is in good shape needs only the most cursory inspection. Traditionally, agriculture is the country's strong suit; growing crops in arid regions is one of the exportable technologies developed, veterinary medicine another. Since 1945, biomedical research has flourished, largely at the urging of Lord Florey and Sir Macfarlane Burnet. So too has astronomy, partly because of Australia's geographical position in the Southern Hemisphere.

What follows is a kaleidoscope of impressions gathered on a rushed journey during June. By being selective, it is necessarily unfair except in the sense that the random citation of a few high-quality projects may prove that there is much more of the same unmentioned.

Australia is strong in the application of *in vitro* fertilization to childbirth. Dr Carl Wood, professor of obstetrics and

gynaecology at Monash University, and Dr Alan Trounson, direct one of the centres. Wood says that his work is a natural extension of veterinary developments in Australia. He reckons that throughout Australia, there are 10,000 women waiting for treatment, more than 2,000 at his own clinic.

Melbourne is a medical powerhouse, with two important medical schools and a traditional backing of outstanding public hospitals. The university has also become one of the several centres of biotechnology in Australia, since when its botany department has been singled out as a centre of excellence by the Tertiary Education Commission. Dr Adrienne Clarke, head of the centre, says she decided deliberately to spend half of her \$A2 million grant on mass spectrometry equipment. Partly as a consequence, her laboratory has become even more the hub of collaboration on the

molecular basis of plant cell physiology.

Throughout Australia, specialized institutes such as the Hall and John Curtin institutes and university departments are turning as quickly as they can to molecular biology. So is CSIRO, partly on the strength of a report by a committee under Dr N.K. Boardman in 1979.

CSIRO's Division of Molecular Biology in the North Ryde suburb of Sydney is one of the smaller divisions, with a professional staff of 48, roughly one-third of them paid for by contracts with outside organizations. How does such a laboratory cover the ground?

Dr G.W. Grigg, the division chief, is a little alarmed that too many of his bright people will devote themselves exclusively to practical projects, making the laboratory less effective at its indispensable role as an impartial source of advice for Australian industry at large. In practice, the

laboratory has already helped to spawn a substantial company, BIOCLONE, an international supplier of monoclonal antibodies specific for pituitary hormones.

Sydney houses a clutch of important CSIRO divisions, including that for mineral physics which claims to have pioneered remote sensing in Australia. Among other things, the division is using magnetotelluric techniques for mapping oil reservoirs, and claims the first systematic application of palaeomagnetism to the study of ore bodies. But its practical product is an instrument called SIROASH which uses nucleonic techniques to measure the bulk ash content of coal; the underlying phenomena are gamma-ray scattering and electron-pair production.

Sydney is also one of the centres of Australian astronomy, largely because of the long collaboration between the department of physics at the university and the CSIRO Division of Radiophysics. Sydney physicists are the prime movers in the plan to build the Australia Telescope, a \$A25 million radio interferometer consisting of an array of six 25-m dishes strung out along a track 6 km long.

This ambitious project is the responsibility of the CSIRO Division of Radiophysics, which believes it will be able to engineer an instrument whose frequency coverage, accuracy and sensitivity will be without precedent. The radiophysics division hopes that its expertise in the design of antennae feeds will contribute to the success of the equipment.

Optical astronomy is also prominent, the flatness of the ancient continent of Australia notwithstanding. The Mt Stromlo Observatory (40 minutes from Canberra), part of the research school of physics at ANU, claims to be the one of the most influential sources of professional astronomers in the world. Its facilities are remarkable, but mostly to be found 500 km to the north, at Siding Spring, which is also the site of the Anglo-Australian telescope.

The latest acquisition is a home-designed 2.3m telescope built at a cost of \$A3.2 million and opened in March 1984. Dr Don Mathewson, director of Mt Stromlo, is especially proud of three features of the project — the fact that the six-inch thick mirror blank (thin as these things go) was fashioned by sawing a Corning blank in half laterally, the altazimuth mounting of the telescope and the fact that the building in which it is housed rotates as a whole on its axis.

The Anglo-Australian Telescope is similar kettle of fish. Dr Dan Morton, the director, reckons that Australian astronomers profit more than British from the facility. As of last month, nobody at the office had been informed officially of the decision by the British Science and Engineering Research Council that continued support (£1 million a year by each side) did not come high on the research council's list of priorities. People in Sydney

say that the agreement to operate the telescope is embodied in a formal bilateral treaty between the governments concerned, and that it cannot be abrogated by the agent of one of them. The next few months should be interesting.

Australia's interest in astronomy has also in the past few years been furthered by the plan, now aborted, for a tripartite collaboration (with the United States and Canada) to operate a 1-m reusable telescope package from a low orbit about the Earth. In Canberra, they have no doubt that the system would have been more economical than the Hubble Telescope.

So Mt Stromlo is now hoping that something will come of another plan, involving both the US National Aeronautics and Space Administration and the European

Space Agency to launch a far ultraviolet detector as part of the Fuse/Columbus project.

This is the conventional part of the exploration of the world outside the Solar System; the radical part is the experiment, based on the physics department at the University of Sydney, by means of which an attempt is being made to put flesh on the bones of a proposal due to Professor R. Hanbury Brown to build large-scale Michelson interferometers from which to extract the diameters of distant stars and galaxies. The department is now bringing into commission an interferometer with a baseline of 11.4 m. Then it will set out to raise more than \$A2 million. Either way, like much else that happens in Australia, the instrument will be unique. □

ASTEC

Vehicle for wise council



THE most remarkable component of the Australian government's machinery for the administration of science and technology is the Australian Science and Technology Council (ASTEC), a device by which independent scientists not only give advice when asked but have a kind of prescriptive right to comment on government policies with technical implications. The closest analogue of ASTEC is historical — the US President's Science Advisory Council (PSAC) in its heyday of the 1950s. PSAC, alas, had lost much of its influence by the time it was abolished in 1971; ASTEC, on the other hand, was invented less than a decade ago and was given a statutory place in the machinery.

ASTEC is not a substitute for a Minister for Science; there is one of those, Mr Barry Jones. Nor is it the minister's creature; rather, it reports directly to the Prime Minister, now Mr Bob Hawke. The council itself, now eighteen strong, is a roughly equal mixture of academics and users, mostly from industry. The chairman is, Professor R. O. Slatyer, director of the School of Biological Sciences at the Australian National University.

Unlike science advisory committees elsewhere, ASTEC is adequately staffed. The secretary, Dr B.D. Middleton, emphasizes that ASTEC's distinctive role is its oversight of the whole of government business (for which reason the organization has access to cabinet papers). Often, he says, ASTEC has persuaded the government that a proposal given only low priority by a minister should be given more serious attention. ASTEC claims some responsibility for the decision a few years ago to re-equip the Bureau of Meteorology and to contribute to the international seismic network for the surveillance of nuclear explosions.

An important part of the philosophy of ASTEC is that its members work hard (they are now paid \$A11,000 a year). Last month, they were collectively finishing off

a round of visits to all the 40 divisions of CSIRO as part of a major enquiry into the role of research in Australia.

By definition, ASTEC cannot avoid being part of the political process. So much is clear from the review of Australian uranium policy, published a year ago (see p.200). One explicit objective of the inquiry was to consider the adequacy of safeguards on the export of uranium.

It seems not to have damaged ASTEC's standing that the government was unable to accept the key recommendation that Australia should play a fuller part in the fuel cycle than as a uranium supplier.

ASTEC seems also to be capable of political initiatives. The government's announcement (in May) that from the beginning of the next financial year (1 April 1986), expenditure on research and development by private companies will qualify to the tune of 150 per cent as a business expense had its origins in ASTEC's glooming about the low level of expenditure by Australian companies.

The present inquiry about the function of research in Australia is a different kind of nettle. The Hawke government's determination that almost every public institution should be reassessed suggests that the question would inevitably have had to be grasped at some stage. That CSIRO should be singled out for the first and most contentious chapter of this crucial examination suggests either that ASTEC has woefully accepted terms of reference that prejudge the outcome, or that ASTEC has preconceived ideas about the location of buried bodies. The best guess is that the truth is neither extreme.

None of this is a complaint. In the past year or so, ASTEC has tackled subjects as different as the place of Videotex in Australia and technology's role for the handicapped. Part of the reason for its influence is that it will deliver what ministers (of whatever party) need. Now and again, it may be able to stir the pot. □

Government's role

Minister with his own vision



In a system of parliamentary government, in which the officers of the executive are chosen from among the people's representatives, every government needs one minister like Australia's Minister for Science, Mr Barry Jones. Whether it could live with two like him is another matter.

Mr Jones, less of a minister than a prophet with political pretensions, is known to both those who have never met him and his enemies (who abound) as "Barry". Barry, in the argot, rendered Prime Minister Bob Hawke a signal service before and after the election of March 1983. Single-handedly, he has made science and technology into an issue that ordinary people take seriously. Nowhere else, except possibly in India now and the Soviet Union in the 1930s, has the exploitation of science and technology been as proper a subject of polite conversation as it has become in Australia.

Mr Jones first became a national figure as an astonishingly successful competitor in the television quiz shows of the 1960s. People still tell of the occasion when he proved that the television network and not he had given the wrong answer. Long memories, of course, do not necessarily make for congenial politics.

Mr Jones has a sense of duty and is considered to be on the left-wing of the Labor Party. He is the author of *Sleepers Wake!*, a tract which, in 1983, sought to persuade Australia that it must use and live with technology if it is to hold its own.

Mr Jones has kept talking about the need for change consistently since taking office in March 1983. In the process, he has popularized the problem of technological change and also helped to confirm his own popularity. But there have been two substantial set-backs. The first, just a year ago, was a shock to those of Mr Jones's supporters who may have thought he was about to wave a magic wand; in the event, the funds for providing university research grants (see p. 194) did not increase by the promised 10 per cent but only by the amount of inflation.

Explanations of what went wrong differ. Some say that the minister brought the trouble on himself by putting increased science spending a long way down his list of priorities, certainly after his proposal for the Commission for the Future (not another exercise in futurology but an attempt to increase Australia's capacity for change). Some think he was playing a devious game by counting on the government's commitment to ensure that the increase came through. The most likely explanation, however, is that the meagreness of the increase may have been a reprisal for Mr Jones having fought within the Labor caucus for complete withdrawal from the uranium mining industry.

Mr Jones's second setback came only after the general election last December, when the Hawke government was returned with a reduced majority. The technology part of the science and technology portfolio was whipped away, and handed to Senator John Button, previously at the industry and commerce ministry, one of the government's strong men and (unlike

CSIRO's industrial might

INDUSTRY has always been an important component of CSIRO's terms of reference. The novelty is Australia's new-found conviction that it can no longer rely on agriculture and minerals to balance the cost of the products its people must import. CSIRO seems eager to play its part.

So the organization now has a fully-owned commercial subsidiary to help with the exploitation of innovations arising in the divisions and called SIROTECH. (SIRO is CSIRO's way of referring to its role as originator in projects as different as SIRONET, a computer network, and SIROSMELT, a small-scale but efficient way of extracting base metals from ores.)

After only 18 months, it is too soon to know how effective the new company will be. Its terms of reference allow it to become the manager of intellectual property, patents and the like, arising from research at the divisions. The company can also invest in "pre-development" work.

Meanwhile, division chiefs seem still to have much of the responsibility for the exploitation of their people's work. Dr G.W. Grigg at the molecular biology division at Sydney reckons to have been the one who

Mr Jones) also a member of the Cabinet.

The reasons for the change seem to have been administrative rather than political. Just as he is not cast in the standard ministerial mould, so he is by everybody's consent except his own a poor administrator. Priorities change too often: he creates a sense of rush when civil servants prefer serenity. Serenity is not Mr Jones strong suit. Much of his personal popularity stems directly from his open friendliness. He is almost too open to be a successful politician, and in any case, he is too imaginative. □

persuaded a Sydney businessman to invest in the production and marketing of a range of monoclonal antibodies. Dr J.R. Anderson at the materials science division at Melbourne worries on a day-to-day basis about the zirconia ceramics project.

Except in agriculture and minerals, Australia has traditionally been a protectionist economy. One consequence is that the Australian manufacturing sector is neither export-conscious nor compelled, by the international market, to be as conscious as would be wise of quality.

So innovators are faced with a dilemma: should they license their bright new idea to an Australian company, knowing that they can help guide the development of the product through the critical stages and earn a substantial royalty on sales at the end, or should they sell the licence to some company overseas, earning a smaller percentage on what may be a larger volume of sales?

Hitherto, political considerations (not to mention the character of CSIRO) have forced local licensing policies on the organization. Will SIROTECH break this pattern? □

CSIRO

Time for thinking again



FOR much of the period since the Second World War, the name for Australian science has been CSIRO, short for Commonwealth Scientific and Industrial Research Organization. During most of that time, the organization has ridden high, rightly boasting of its splendid achievements in agricultural research, innovative work in basic research and of being the chief custodian of Australia's international reputation in research.

But now the gilt is flaking off the gingerbread. The universities are stronger than they used to be, while government is increasingly concerned to know what benefits it gets for all the money (\$A382 million in 1983) and the services of all those people (more than 7,000 staff, over 2,000 of them professionals). So CSIRO is on the defensive. ASTEC (see p.188) has been asked to inquire into the entire role of the research enterprise in Australia, but to give priority to its views on

CSIRO, which is conscious of having blotted its copybook with the new government and which is responding a little frenetically to the clamour that research should benefit manufacturing industry.

Some in the government do not forgive CSIRO for the letter sent by its chairman Dr Paul Wild to the incoming government asking that the organization should be transferred from the Department of Science and Technology; the letter went off before the cabinet had been sworn in, and so was delivered to the out-going Fraser government, irritating both camps.

But even those with the shortest memories recall the great row over the Australian National Animal Health Laboratory at Geelong, near Melbourne, partly because of the high cost (\$A157 million) but mostly because the plan to import live foot-and-mouth disease virus had not been discussed with a quarantine-conscious agricultural community.

Farmyard computers and biotechnology

At the CSIRO Division of Plant Industry it is clearly passé to ask what impact molecular biology is having on agriculture. People have stopped thinking of it as a subject; it is rather a set of tools which everyone uses.

There is no doubt that the division is a dynamic one and that it has a dynamic boss in its director Jim Peacock. One suspects that a man who became director of a plant industry laboratory by working on *Drosophila* will have no difficulty in persuading government of the value of whatever he feels should be done.

Balance between basic research, "strategic mission-oriented research" and short-term applied research is maintained at 20:20:60 per cent. But there is clearly a premium placed on generating new concepts for agriculture from basic research (such as the developments of diagnostic kits for plant viruses) rather than just "agricultural improvement".

It is not only molecular biology that is taken for granted in the division; computers have also arrived. One hundred million dollars worth of the annual cotton crop is now grown to a tune sung by a computer-

based management system, SIRATAC.

In the SIRATAC system, years of data collected on crop growth, insect pests and insecticide effectiveness are built in. Every few years the farmer inputs on his own terminal the numbers of insects on his cotton and the numbers of fruit; the central computer adds weather data. The program then predicts from the likely effects of weather, beneficial insects and previous sprayings the mortality of fruit and the number likely to survive to harvest. This is not so simple as more fruit are produced than will mature; light insect damage might even increase yields by removing unnecessary fruit.

Beyond SIRATAC come systems for other types of irrigated agriculture. SIRACROP is now poised for commercial development and will decide irrigation schedules for farmers growing wheat. And beyond that again comes a total system for a range of crops — soybean, maize and the like — which will not only increase farmers' profits but also stop the poorly managed use of water contributing to rising water tables.

Alun Anderson

One casualty of this distrust seems to be Dr Wild, the chairman, whose term of office ends in September, and who will be replaced for nine months by Dr Keith Boardman, formally due to retire as a full-time member of the executive of CSIRO on the same date. Dr Wild, previously director of the Division of Radiophysics at Sydney, and the driving force behind the distinctive Australian system for landing aircraft automatically, is generally thought to have been poorly equipped with the mixture of guile and directness required for success in Canberra. CSIRO is unusual among the descendants of the organizations set up in the 1920s in what the British call the old Commonwealth. Its terms of reference cover everything from agriculture to minerals extraction and metal processing to general support for manufacturing industry. Two years ago, CSIRO adopted a self-denying ordinance on medical research, agreeing that it would not undertake clinical research except in collaborations.

CSIRO's structure is as unusual as its scope is unbounded. The chief executive is the chairman, appointed by Canberra, who has two full-time fellow members of what is called the executive, on which day-to-day decisions fall. There are also five part-time members, whose function seems to resemble that of the non-executive members of corporate boards — to act as informed referees. Key decisions rest with this handful of people. There is also an advisory council whose function is not so much to participate in decisions as to interject opinions.

Part of the more obvious trouble is that this band of men, housed in a new piece of modern concrete half-way to the airport at

Canberra, is necessarily a long way from where research is practised. Day-to-day matters take precedence: thinking ahead is harder. The remoteness of the executive has been enhanced by an earlier reorganization, in 1972, when individual laboratories (called divisions or sometimes units) were grouped into five bundles, called institutes, each with a director of its own. These, too, appear to be condemned to too great a burden of paper-pushing. The real directors of research are the heads of the divisions (of which there are 44), known as "chiefs" and addressed by their colleagues by that word. Typically, a chief will be a man in his fifties, often one with scientific distinction overseas, but who will usually volunteer that his chief function is to provide an environment in which younger people can be creative.

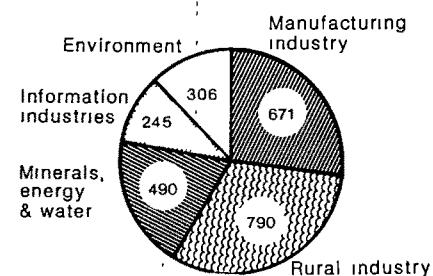
Scale is more difficult. Typically, a division will have about 50 working scientists, formed into perhaps half a dozen research groups. Some division chiefs admit that their laboratories are big enough only to make a few distinctive contributions to the international enterprise so as to earn the right to keep a watching brief on what is happening elsewhere.

The quality of the work seems nevertheless to be uniformly excellent. So why do Australian governments not cherish CSIRO as in the old days? Part of the explanation is that there has been a change of government. Although discontent has been rumbling for many years (witness the 1972 reorganization), and while the Hawke Labor administration is in no sense a doctrinaire socialist government, it was elected partly on the strength of Mr Barry Jones's plea that Australia should prepare to join the twenty-first

century (see p.188); it was natural that CSIRO should have felt threatened by its arrival.

Some of the accusations made against CSIRO are nevertheless unfair. Thus it is widely held that the organization is not, or not sufficiently, accountable. Unlike many other research organizations, however, CSIRO is not autonomous. Quite apart from the familiar mechanism of control by annual budget, CSIRO's enabling act (of 1949) gives the government the right to issue directions to CSIRO. Internally, CSIRO operates a system of periodic reviews of its divisions, the consequences of which are often the amalgamation or reorganization of laboratories, or substantial changes of the emphasis. If accountability at the bench means a sense of people looking over one's shoulder, CSIRO is blameless.

CSIRO is, however, vulnerable to the complaint that it is ingrown. Unusually, it is a career research organization. During the past quarter of a century the supply of home-grown research scientists has grown from a trickle to near-sufficiency. CSIRO has become a substantial source of academic and industrial scientists, but its core staff has become over-used to what many think of as the traditions of the service. Bringing in people from outside, especially to senior posts, could not fail to help.



CSIRO's research effort by purpose and/or beneficiary, 1983-84. Numbers indicate professional scientists engaged. Source: CSIRO Annual Report.

CSIRO is also large, in Australia, conspicuously so. The central administration of close on 50 establishments and the wise arrangement of objectives in some coherent order would tax the most enlightened organization. The concentration of responsibility on the shoulders of three full-time executives seems to predetermine both the appearance of stubbornness and that of irresolute compliance to outside pressure.

The terms of reference are in some ways an encumbrance: "... to plan and carry out a comprehensive program of research on behalf of the Commonwealth for the benefit of the people and industry of Australia". Research policy is thus not easily distinguished from public fashion.

The present policy (see *Annual Report, 1983/84*) is to transfer to seven fields (biotechnology, advanced materials, manufacturing technologies, information technology, water and soils, plant pathology

and oceanography resources that can be quarried from elsewhere by natural wastage (3.5 per cent a year).

The recent growth of numbers of those working in these fields is impressive evidence of how a determined executive can make things happen; the danger is that the interest of continuing research in both minerals processing and, more particularly, agriculture, will be undervalued. The importance attached to biotechnology may have agricultural implications, but there seems to have been only scant consideration of the continuing need for research in agriculture, or even that when markets turn sour, the need for research may be all the greater.

The other side of this coin is that CSIRO may have less to offer industry than it believes. Historically, CSIRO's specific terms of reference have included responsibility for "strategic research" in support of technology. It is also the undisputed case that many divisions have been the source of valuable industrial innovations, and that Australian companies are too neglectful of the value of research and development. But while there is no distinctive economic pattern in Australian manufacturing industry, at least from the standpoint of the export markets, it is hard to see that CSIRO's efforts to reinvigorate the manufacturing sector can do more than spread resources thinly over unknown territory.

Certainly there are many Australian industrialists who complain that CSIRO should not harbour ambitions in this field. Thus Dr Lou Davies, research director of the electronics and defence manufacturer AWA, has publicly said that CSIRO should stay in the patch it knows. Among the division chiefs, who seem to accept the new policy willingly, there seems some confusion about the degree to which basic research, whatever its potential value to manufacturing industry, is permissible in the new regime.

These difficulties could be enormously simplified. Outsiders are bound to ask why a felt need to improve the research base of manufacturing industry should require the retreat from agriculture and minerals. The questions would be more easily decided if there were three distinct organizations for agriculture, minerals and manufacturing industry.

Dismemberment is the least preferred solution at CSIRO, which is natural enough. Fair play, there are other steps that might be taken to make the organization less vulnerable, among which a revision of CSIRO's management structure seems the most obvious.

Over the years, the troika which is the executive has become the day-to-day management; the influence of the part-time members varies, but often requires that able scientists should be involved in the choice of priorities for the years ahead (at which they are not necessarily skilled). It could not but help if this group of people

functioned more like a board of directors of a company, not (as they sometimes appear) a rubber-stamp.

It is hard to think that in such an environment, universities would continue to occupy as marginal a place as they do at present in CSIRO's thinking. The days (in the 1950s) have long since gone when universities were dwarfed in competence by CSIRO. Yet CSIRO does not yet behave as if its remit "to plan and carry out a comprehensive programme of research . . ." could be read as an invitation to place major research grants within university departments. The benefits would

be flexibility, perhaps even lower costs but also the further blurring of the old patronizing relationship between the professional researchers and the teachers.

None of this is to suggest that CSIRO is indifferent to university problems. The physics department of the University of Sydney has succeeded largely because of its past collaboration with the Division of Radiophysics, while the planned Australian Telescope will be a great asset for university groups throughout Australia. But by being more guileful, might not CSIRO also be more effective, and have more friends?

John Maddox

Walter & Eliza Hall Institute

Towards a malaria vaccine



THERE seems no stopping the Gus Nossal show, the Walter and Eliza Hall Institute of Medical Research at Melbourne. At the end of last month, the institute was given by the government one of its largest ever grants — more than \$A6 million over five years for its contribution to the development of a vaccine against malaria. In keeping with the spirit of the times, the government's Industrial Development Board will have a right to 40 per cent of the value of the intellectual property that emerges.

That the Hall institute, technically an independent charity, should win a commanding place in Australian biomedical science is largely the doing of two successive directors, Sir Macfarlane Burnet (1944–65), a 1960 Nobel prizewinner, and now Sir Gus Nossal (since 1965). But being in Melbourne helps. Melbourne medicine, concerned though it may be with the curing of the sick, is also anxious to dominate Sydney, and so is warmly supportive of its own institutions.

Burnet, at 85, is as chirpy as ever. An immunologist before molecular biology came of age, he gave the Hall institute intellectual standing at a time when Australian science was regarded as derivative of that in the United Kingdom. He denies that he has dragged his feet on molecular biology, in his book *Genes, Dreams and Reality* (1971), but argues the need that researchers should be patient. His small-talk (of which there is not much) tends to cosmology as much as immunology.

Nossal is more ebullient, much more the leader (and driver) of men. Austrian by birth, and a protégé of Burnet, almost all his professional life has been spent in Australia. (There were two postdoctoral years at Stanford.) The Hall institute is still small enough (with a staff of 200) for Nossal to continue to function as director of the cellular immunology unit at the institute, one of the eight between which the institute's work is divided.

The most tangible sign of the institute's success is the new building, financed

largely by the governments of Australia and Victoria, into which Nossal hopes to move later this year. One of the institute's frustrations is that the building is at least a year behind schedule, largely because of labour troubles during construction. Nossal says that some features of the design were incorporated into the building at Cambridge, Massachusetts, in which the Whitehead Institute is now housed; he finds it galling that a building on which \$A20 million has already been spent should be so far behind.

The move to the new building, 100 metres away, has been made necessary by the growth of the past few years, and by the need of space in which the institute is now housed. Until the latest grant was announced, the malaria vaccine programme accounted for roughly a quarter of the whole programme, whose cancer research unit has now become a Ludwig institute.

Nossal's operating costs are low by US standards but higher than in the United Kingdom. Last year, the institute spent \$A7.3 million, of which more than a half (\$A3.7 million) came from the federal government's grant-making agencies, chiefly the National Health and Medical Research Council. The Victoria government contributes \$A500,000 a year, while Nossal is proud last year to have pushed the value of grants and contracts from overseas above \$A1 million for the first time (with the help of the Rockefeller Foundation and the US National Institutes of Health).

The malaria vaccine programme is inevitably the most glamorous part of the institute's programme, although Nossal says (before the latest grant) that it accounts for only a quarter of the total effort. In reality, the present excitement is the product of ten years of development at the Hall institute, and malaria (particularly that caused by *Plasmodium falciparum*) is only one of the half-dozen or so parasitic diseases in which the unit has an interest.

The present objective, based on a

knowledge of the genome of *P. falciparum*, is to develop recombinant antigens characteristic of the parasites that may be expected to stimulate the immune response when injected into people. The key development (see *Nature* 306, 751-756; 1983) was the demonstration that antigens characteristic of the blood-stage (merozoite) parasites can be expressed in *Escherichia coli* and that surface antigen, itself a molecule with relative molecular mass of 200,000, contains a repeating unit of eleven amino acids. Since then, the parasitology group has been able to show that peptides derived from naturally occurring *P. falciparum* proteins and containing even shorter repeating sequences of amino acids can react with immune sera (*Nature*, 310, 789; 1984).

So the way seems clear for a systematic programme of work with a high chance that it will lead to the means of immunizing people against malaria parasites carrying the particular antigen investigated. Robin Anders, head of the malaria team at the Hall institute, is nevertheless eager to emphasize the difficulties ahead. The extent of the variability of the antigens between the different strains of just *falciparum* is not yet settled, while the task of working through the genomes of the other species of *Plasmodium* responsible for human malaria has hardly begun.

Another difficulty ahead is that an effective human molecular vaccine will almost certainly have to stimulate immunity against at least two other antigenically distinct forms of the parasite, that carried in the liver and the sexual stages transmitted to the mosquito vector. As a consequence, Dr G. F. Mitchell, head of the immunoparasitology unit at the Hall institute, says that there is now close collaboration with other groups working on these stages, the Nussenzweigs at New York University for example.

Even before the \$A 6 million grant from the Australian government was announced, Nossal was optimistic about what lies ahead. He reckons that it should be possible to tell what the vaccine cocktail should contain and to have laboratory evidence of its efficacy within three years, and that a working vaccine may be used for the first time in five years or so. Nossal is also full of praise for the WHO Special Programme of Research and Training in Tropical Diseases, whose existence coincides with that of the Hall institute's explicit interest in parasitology.

For the rest, the laboratory is up to its neck in cell biology of all kinds. Nossal says there is virtue, nowadays, "in being able to call almost anything immunology". Nossal's own group is engaged on the hunt for the chemical messengers between lymphocytes which are presumed to modulate the immune response, generically the lymphokines; one of its more recent successes has been the characterization (down to amino-acid sequence, in

collaboration with Dr Lee Hood's group in California) of the P-cell stimulating factor (PSF), thought to stimulate the proliferation of haematopoietic cells when released by activated T cells.

On the principle that immunology spans all cell biology, the Hall institute is inevitably involved with questions such as the maturation of T cells in the thymus. (The local doctrine is that only a minority of

thymocytes eventually survive as T cells.) The translocation of the *myc* gene in oncogenesis is just as inevitably prominent in the research programme, but the promise for the future may lie in the programme of the molecular biology unit, which is concentrating on the relationship between oncogenes and growth factors (and the receptors for them) in the causation of cancer. □

Universities

Entangled in red tape



THE universities of Australia are in a state of flux, but that is nothing new. Since the 1940s there has been an almost continuous examination of the function and operation of educational institutions, both universities and the Colleges of Advanced Education (CAE), many of which have evolved from the old state-run teachers' training and agricultural colleges.

The consequences of this selfconsciousness have been beneficent. The system has grown, and is still growing. Among the nineteen universities proper, there is an interesting diversity, neatly illustrated by the differences between, say, the James Cook University of North Queensland (p.195) and the University of Adelaide (p.198). Since the Second World War, the universities as such have also been transformed from undergraduate teaching institutions to institutions with substantial and effective research programmes and graduate schools. So why is there persistent anxiety about the working of the system?

The arrival of the Hawke government in March 1983, with its commitment to more open access, was inevitably a spur to further introspection. There are three sets of issues in the air — budgetary problems (including the question of whether students should be required to pay fees, as used to be the case before the 1974 Whitlam government), support for the direction of research and the management of the system as a whole.

The pursuit of administrative tidiness over the past decade in particular has complicated the management problem. Professor David Caro, vice-chancellor of the University of Melbourne, has said that the administration of higher education in Australia "is the most complex in the world... no other country... has invented such an involved co-ordinating and advisory apparatus".

This is how it works. The Australian universities (nineteen plus the Australian National University at Canberra) are supervised by the Universities' Council, whose existence goes back to the recommendation of the Murray committee in 1956 that Australia should interpose a council similar to the British University Grants Committee between government and the universities; the complication, in

Australia, is that there are two tiers of government, federal and state. Since then, two further higher education councils have emerged, one for CAEs called the Advanced Education Council and one for Technical and Further Education (TAFE). The notion that each of these councils should make separate claims on federal and state government funds was recognized by 1974 to be a recipe for conflict, which the Whitlam government sought to rationalize by creating a Commission for Tertiary Education to coordinate the work of all three councils. The commission came into being only in 1977; the delay in part is explained by the dissolution of the Whitlam government by the governor-general, Sir John Kerr, in 1975.

What now seems clear is that even the new system is unworkable. The newly appointed chairman of the Commonwealth Tertiary Education Commission (CTEC), Mr Hugh R. Hudson, puts the point eloquently in a document published earlier this year, a response to the federal government's request for yet another review of the administration of higher education.

Before 1974, the federal government contributed only 35 per cent of the public subsidy of higher education, the remainder coming from the states, but the Whitlam government (with the agreement of the state governments) took financial responsibility to itself in 1974. The result, according to Hudson, is that the states support "the sometimes unrealistic claims of the institutions" in their territory. He says that in the bidding for funds for the three-year period beginning this year, one university, apparently with the support of its state minister of education, asked for a capital sum of \$A100 million, close on two-thirds of total federal spending on capital development in higher education during the previous three years.

Administratively, the present system is also cumbersome. Each council is expected to produce a coordinated bid for federal funds which is then coordinated into a unified submission by CTC. In effect, Hudson says, much of the work is done twice, the volume of paper delivered to the government tends to confuse by its bulk and contradictions and, in any case,

CTEC's supposedly coordinated advice has often to be submitted before the three subsidiary councils have finished their own documents.

Hudson's complaints will be generally accepted but his proposed solutions are controversial. Hudson would turn all three subsidiary councils into advisory committees, concentrating responsibility in his own CTEC; both the universities and the state-oriented TAFE sector are against the proposed change, although the CAEs appear to think they would gain either from that or from an amalgamation of their council with that representing the universities.

Hudson is less clear in his proposals for changing the federal/state relationship. He asks that states should determine their own priorities for the relative development of the three sectors, which is sensible enough; he also says that CTEC should itself have close relationships with universities and CAEs, which need not prejudice the present sponsorship of universities and CAEs by the state governments; but he shrinks from considering whether there should be some change in the present division of financial responsibility.

Two related themes run through Hudson's ambition for the system — the need that socially disadvantaged groups of young people (immigrants, called "migrants", and aborigines) should have opportunities in higher education, and the need for greater cooperation between the three sectors. The first objective is widely accepted, by the government no less than by individual institutions. The second will raise more serious difficulties, even though there are already several institutions that span the boundaries; Hudson points out that James Cook University offers courses appropriate for CAEs, and that the Royal Melbourne College of Technology provides teacher training as well as TAFE courses. But Hudson is also looking for arrangements under which credit can be given for courses which can then be transferred elsewhere.

Hudson's plan would also transfer to CTEC responsibility for ARGs (p.194). The argument is that there is now no assurance that those winning grants have the resources to use them effectively, that the result would be greater efficiency and economy, and that CTEC could better assess the need for an increase in ARGs funds than would the science department.

The Hudson prescription for administrative reform is certain to be influential; the government will have to respond to it before the end of the year. But the plan to take over ARGs, one of Hudson's admittedly "more distant" goals, is also thought to be his own negotiating ploy, something that can be given away later on. The more serious question hardly ever raised is whether institutions, and universities in particular, might be liberated from the sense of detailed remote control from which they now suffer. □

Students and budgets hit plateau

THE growth of Australian universities over the past decade has been dramatic but has now stagnated, as have the budgets of individual universities. Many now argue that, without saying as much, the federal government is requiring the universities to do more with less.

Student numbers are now some 18 per cent greater than a decade ago (at roughly 170,000). Within this total, there has been a widely welcomed increase of the proportion of women (up from a third to 45 per cent). Since the abolition of university fees in the 1970s the most dramatic growth has been in the numbers of part-time students in the university population: more than a third of undergraduates and 60 per cent of those studying for higher degrees are part-timers.

The universities differ among themselves both in size and in the general character of the courses they provide. The University of New South Wales (mostly in Sydney) is the largest, with 19,000 students, with the Universities of Sydney (18,000), Melbourne (16,000) and Queensland at Brisbane (18,000) not far behind. But even some quite new universities, such as Monash (founded in 1958, now with 14,000 students) and Macquarie (founded 1964, with 11,000 students), at Sydney and Melbourne respectively, have been able to grow rapidly. At the other end of the size scale are James Cook and Murdoch (in Western Australia), with 2,000 and 3,000 students respectively.

During the past five years of near-stagnation, the general response of the larger and thus more flexible universities has been predictable — to concentrate teaching and research on the stronger departments, closing down those that have a lesser contribution to make, and to seek external income by means of research contracts. The decisiveness with which the larger establishments have been able to respond to these pressures would delight the

British University Grants Committee, which for similar reasons in different circumstances is asking for exactly these responses.

In Australia, the Universities Council (one of the three statutory components of CTEC) has done what it can to strengthen good departments by persuading the universities to found ten "Special Research Centres", intended to be centres of excellence within the university system as a whole. (One of the ten centres, a nerve-muscle research centre at the University of New South Wales, was closed soon after being founded in 1982.)

The conditions under which these centres have been founded are exceedingly flexible; within reason, those appointed as directors are able to spend the funds available (up to \$A10 million over five years) on staff and equipment or some blend thereof. Academics and their critics appear to agree that an extension of this scheme would be valuable.

There is much less agreement about steps recently taken by the Universities Council to ensure the efficient administration or research funds in universities. The council's chairman, Professor D.N.F. Dunbar, wrote to vice-chancellors two months ago asking that universities should not "distribute by formula" this component of their recurrent budget, and including the ominous statement that "the present approach, which characteristically allows staff to follow their individual interests" is unlikely to make "the best use of the limited funds available".

For what it is worth, the successful universities are already seized of this need, while the successful university departments are fully aware that the success depends on the recruitment of outside funds. It is not for nothing that Professor Harry Messel, head of the School of Physics at the University of Sydney, boasts of having raised \$A30 million in as many years. □

Australian National University

A bed of "tall poppies"

AUSTRALIA's most unusual university, the Australian National University (ANU), is not so much a university in the ordinary sense as a collection of specialized institutes of advanced study to which there was added (in 1960) an undergraduate teaching establishment fashioned from the pre-existing University College of Canberra.

ANU is financed directly by the federal government, not through CTEC, as are the remaining universities, and for that reason is jealously regarded by academics elsewhere. The jealousy centres not merely on the separate flow of money (\$A135 million in recurrent costs) but on the terms of reference of those ANU academics in the seven research schools (Biological Sci-

ences, Chemistry, Earth Sciences, Pacific Studies, Physical Sciences and Social Sciences), who are required only to prosecute research, and who teach by grace and favour, sometimes at other universities.

Over the years, ANU has turned out to be a powerful means of attracting academics from overseas to settle in Australia. Inevitably, in an egalitarian land where "cutting down the tall poppies" has become the general idiom for the levelling process, ANU has become a focus for controversy. The excellence is not disputed, but academics elsewhere increasingly argue that they have to be excellent to succeed, and have to teach as well.

ANU's defence is twofold: that there is a small army of graduate students, drawn



from all over Australia, and that, in any case, many members of the research schools help out with lecture courses provided for the faculties. But only the research school of chemistry (at the instance of Professor Arthur Birch, the school's first director) is actually contiguous with its undergraduate department.

There is also a certain clubbiness about the relationship between the federal government and ANU. Last month, one member of the university described in a quite different context how he had been able to win support for a project by inviting a minister to a dinner. The research school of chemistry was apparently brought into being at a dinner in London (England) at which Sir Robert Menzies (then prime minister of Australia) and Professor Arthur Birch were both present. If those who would cut down tall poppies can be persuaded to stay their hands, there may be an interesting future.

The other easy complaint to make against ANU is that, not being part of the university system as a whole, it is not subject to the pressures of which other Australian universities are repeatedly reminded, that an academic's first responsibility is to make Australia prosperous. ANU's answer is quick and disarming: we do. And broadly speaking, ANU is right.

The research school of Earth sciences has Synroc to boast about; Australia has high hopes that this will become the world's preferred way of sorting radioactive waste. The School of Physical Sciences has spawned what must be one of Australia's most unusual companies, Optical Fibres (Research), which has received a grant of \$A5.4 million from the Industrial Development Board to develop a range of small sensitive sensors based on the fidelity with which plane-polarized radiation is transmitted uncorrupted through optical fibres subjected to various external conditions. Significantly, this development is an offshoot of the department of applied mathematics at the school.

To a hurried visitor, there is no difficulty in understanding why ANU is attractive to those who work there, or in accepting the claim that the institution has fulfilled the hopes of the university's founders that it would become a creative force in Australian intellectual life.

With the passage of time, and especially as the research schools at other universities have been strengthened, collaboration between ANU teams and others has become prominent, and should grow. To the extent, of course, that the need for such an institution has been diminished by developments at other universities, the question naturally arises whether ANU should continue indefinitely to exist. The simple answer is that it would be unthinkable, even in Australia, to cut down such a tall poppy; the best solution must be to turn the institution into a truly integrated university, eroding the present gulf between teachers and researchers. □

Research support

Catholic scheme for grants



AN academic scientist without a grant is like a magician without a silk hat (from which to produce rabbits); he may be able to manage but there is a danger he will not try. That is one reason for sharing the alarm now voiced annually by the Australian Research Grants Scheme (ARGS), the only source of funds for research in general, as distinct from biomedical research or one of still narrower sectors (marine science, for example) to which the Commonwealth government has accorded priority. Each year, the managers of the scheme (academics to a man, but working to the Ministry of Science) ask for more with a despair that outdoes preceding displays of outrage.

The scheme dates from the recognition 20 years ago that Australian universities had somehow equipped themselves, against earlier traditions, with people capable of research of the highest standard, and of teaching graduate students. In 1965, the federal government thought that £A1 million (then the Australian currency) might suffice, which it may have done. Now the complaint (familiar elsewhere) is that the budget has shrunk to less than half of what is needed to support all worthwhile projects. This year (1985) only a quarter of the new applications judged worthy of support won grants.

Everybody, even the government, seems to agree that this is unacceptable; but then nothing happens.

With appropriate adaptations to Australian geography, the scheme is much like those in operation elsewhere. People apply for grants at the beginning of each year, all applicants are interviewed (by an itinerant subcommittee) and grants are awarded on the recommendation of a committee (chairman, Professor Peter Sheehan, University of Queensland) by the Minister for Science. Applicants may be from any discipline, and need not even be academics (but invariably are).

The committee in charge does not mince words. "Funding for ARGs is dismal", it says in the introduction to the report on this year's allocations, after cheekily quoting Prime Minister Bob Hawke as having said a year ago that his government intended to support "excellence in research wherever possible".

Part of the trouble seems to be that the government's own rhetoric about the importance of research has increased grant applications while the budget has merely kept pace with inflation. This year, there was \$A23.9 million to spend on 2,359 applications that would have cost \$A66 million. As a consequence, according to the committee, 15 per cent of the 1,000 plus projects begun in earlier years had to be brought "prematurely" to an end. In constant money, the present budget is less than that a decade ago. The average

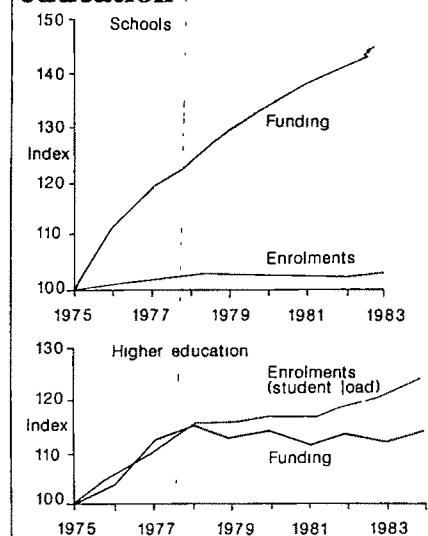
annual value of a grant has fallen to \$A19,478, compared with \$A24,475 (a 1984 price) in 1975.

There is more to these complaints than empire building. The committee considers that its function is to cater for academic research in the round, whence the mixture of programme and project grants, equipment grants and career fellowships (for postdoctoral people). It rightly boasts of having backed a number of important projects, the programme for the *in vitro* fertilization of animals (including humans) among them.

For the years ahead, the committee has identified a number of new directions or which it would embark. Modestly, it asks for an extra £A3 million for equipment for 1986 (to double that spent this year) and money for supporting 30 postdoctoral fellows and 20 postgraduate students (to be attached to the better projects). It would like to be able to offer the ablest applicants a measure of long-term support and asks for \$A500,000 to spend in 1986 or schemes that will help to put Australia on the international intellectual map.

If everybody agrees that ARGs is a deserving cause, and that it is run well, why does its budget grow at such a modest rate? In the climate of Canberra, political explanations are unavoidable. Why should politicians increase the budget of an existing organization and run the risk of being called spendthrift when they can just as easily commit a few million dollars a year to some special scheme (as well as for marine science, there are now special grant-making schemes for biotechnology, energy research and water research) and gain the credit for a new initiative? Surprisingly, nobody complains at the earmarking of funds, but people say that the

Support for higher education



In recent years, the funding for undergraduate education has slipped behind that for schools

multiplication of grant-making committees is a nuisance.

On or just above the horizon, however, there are at least three ways in which the future of ARGS will become a political issue. The most immediate is the demand by the chairman of the Commonwealth Tertiary Education Commission that ARGS should be administered alongside the universities (see p. 192). The argument is that it would be simpler that way. Academics seem to agree with Don Aitkin, professor of political science at the Australian National University and deputy chairman of ARGS, that the demand has been made only to be bargained away in return for some other concession at a later stage. They may have reckoned without Mr G.S. Hudson, the commission's chairman.

The ARGS budget, although less than 10 per cent of that of CSIRO, also touches the credibility of the science minister, Mr Barry Jones. The Hawke government is generally thought to have committed itself to increasing the budget by 10 per cent a year, and for 1984 did better. But last year, Mr Jones emerged from the budget fixing with only 6 per cent extra for ARGS. The outcome may say more about the relationship between Mr Jones and his colleagues than about the government's commitment to science (see page 188), but many see it as an election promise abandoned after victory at the polls.

Moreover, even academics who do not need ARGS or who are prevented from using it (as are those at the Australian National University) think the budget a disgrace. ASTEC has consistently said the funds available for research should be increased. No doubt its forthcoming report on research in Australia will repeat the message, perhaps more persuasively. People relate with black glee the tale of how in 1976 the grants budget was arbitrarily reduced because the then minister had not understood the difference between the financial and the grant-making year, which, they imply, just shows the indifference of governments to research. Unless the government is careful, ARGS could become an issue that divides it from the technical community.

There are alternatives to merely spending more. The Tertiary Education Commission's scheme for endowing centres of excellence (if continued and increased) is a means by which young able people can become established. Even the earmarked schemes can get people started, while it may seem to a government repeatedly made aware of over-academic university systems elsewhere that it may reasonably calculate the maximum restraint is the best policy, at least for the time being. The dilemma it must resolve is whether it makes sense to continue producing potential researchers whom industry will not employ and who cannot pursue their craft with government funds.

The scheme's policies are catholic.

While most successful applicants are at universities, the scheme can be used by people working at, say, state museums. Emeritus professors have a chance. The committees try to concentrate on certain fields (such as ionospheric physics or the mathematics of category theory, pioneered at the University of Sydney).

Grant applications reflect laboratory housekeeping needs. More than 60 per cent of the total sum awarded goes on salaries (for research assistants), less than \$A1 million (in 1985) is for applicants' travel, almost all of it within Australia.

Among the scheme's university clients,

successful researchers say that awards are generally too small, and do not obviate the need for supplementary funds from elsewhere. There is also a certain amount of grumbling by people in the natural sciences that the humanities and social sciences should consume nearly 20 per cent of the total, and on the other side of the fence, shock-horror at the sums spent on physical equipment. But the committees awarding grants insist that they work well together, and that ARGS should not be split. A better course would be to bring all the government's research grants schemes together. □

James Cook University

University of the Tropics



ONE of the occasional hazards of the James Cook University is to come in in the morning and find seven feet or so of python wrapped around a pipe in one's laboratory. The trick, apparently, is not to panic but to unwind it (or even better, have someone else unwind it) and put it somewhere useful, which according to Professor Campbell, of the Tropical Veterinary Science School, would best be the hay-shed, where it can perform the useful service of keeping down the mice.

Pythons, and some other snakes on which it would be singularly unwise to tread, well demonstrate what foreigners sometimes forget: that much of northern Australia lies firmly in the tropics (Australians simply forget that anybody lives there). Townsville, where James Cook University is located, is actually just 19° south of the Equator; close by are both arid tropical areas and tropical rain forests hardly different from those of Java. These environments, plus the Great Barrier Reef — 2,500 or so reefs stretching 2,000 miles along north Queensland's coast, with Townsville lying roughly at the centre — give James Cook University its special

character. It is the only university in the developed world that lies in the tropics, and has immediate access to tropical ecosystems.

The university itself is quite small, with just 3,000 students, but it has a unique facility in its tropical veterinary graduate school, set up in 1969. Against a background of declining university expenditure, the school has been able to expand steadily as demand for trained researchers in animal health and production has grown throughout the tropical world. At present, there are 60–70 graduate students with almost as many from South-East Asia as from Australia. There are close links with Indonesia and some 14 scientists work full-time at a research institute for animal diseases set up by the university at Bogor, with funds from the Australian Development Aid Programme.

Of major significance is their work on the filarial disease onchocerciasis, which in humans can cause blindness. Because filarial disease is also widespread in local cattle, in which it causes no more than lumps in the muscle, potential anti-filarial drugs are being tested in a pro-



The underwater laboratory developed at James Cook University — sponges are being incubated at a depth of 10 metres.

gramme supported by the World Health Organization.

Elsewhere, everyone seems to be making use of the opportunities offered by the Great Barrier Reef. The biologists, who offer the only degree course in marine biology in Australia, have progressed from completion of coral taxonomies to studies of reef community structure, reproductive ecology and population dynamics. Physicists have been modelling storm conditions, and engineers designing structures to keep out the box jellyfish (sea wasp) which makes swimming dangerous for more than half the year. Altogether, the university has been able to produce well over 100 papers in marine biology, from 11 different research sections, in a single year.

Even the chemists have managed to exploit the reef. Marine animals are an almost untapped reserve of natural chemical products, many of which have pharmaceutical potential. Early work was triggered by the Roche Institute of Marine Pharmacology at Sydney; with that institute's demise, interesting looking chemicals go to a US pharmaceutical company and the thrust of research has turned to finding out why the soft corals are so good at not being eaten by predators. (The answer is that they are full of terpenes,



A novel approach to university architecture at James Cook.

which not only deter predators but can be released to kill nearby hard coral, allowing the soft coral to overgrow it.)

The university even has its own miniature marine institute in the shape of the Sir George Fisher Centre for Tropical Marine Studies, named after the university's first chancellor. Here again, there is interest in natural product chemistry, but with a novel twist — the species studied cannot be kept in an aquarium so the laboratory bench is moved to the sea bed. The centre also keeps the Australian Collection of Marine Micro-organisms, as yet virtually unknown outside Australia, including more than 170 blue-green algae and two hundred bacteria, including petroleum degraders.

For geologists, the university's attraction is not so much the reef, but that half the nation's mineral resources lie north of a line drawn through James Cook, a region with no other university to serve it. To exploit their situation, the geologists have set up an Economic Geology Re-

search Unit (EGRU). In return for an annual membership fee, the hundreds of exploration geologists who roam the north can find a home to analyse samples and consult maps. Although EGRU is essentially one largish room equipped with geological and topographical maps and Landsat images, access is also given to departmental facilities including a scanning electron microscope. The centre has clearly been a success; numerous companies have signed up, which in turn has helped recruit funds for work carried out in the department, providing input into an MSc course in exploration and mining geology.

This neat tie-up with business does not,

however, prevent the head of the geology department, Professor Bob Carter, from pointing out Townsville's big disadvantage — isolation that means political isolation from the decision-making and grant-giving heart of Australia and, more important, isolation from fellow scientists. With 12 full-time staff, 15 PhD students plus a few research fellows and a yearly travel budget of just \$A2,500 (a return ticket to Sydney costs \$A700), the complaint is natural. Fortunately, the reef provides a bit of help. Now that Townsville has an international airport, quite a few scientists on round-the-world trips stop off to see the reef, dropping in at the university on the way. **Alun Anderson**

University of New South Wales

Appealing to industry

SYDNEY'S "other" university, the University of New South Wales, complains like the rest of them at the steady erosion of its budget (by an estimated 2 per cent a year). But it is big enough, with 18,000 students, to be flexible in the management of its affairs. It also has the unique benefit of an agreement with the federal government to operate the Australian Defence Force Academy, more than 100 miles off-campus at Canberra.

Chartered only in 1952 as a technological university, the institution has a flavour of diversity. As well as medicine there are faculties of applied science, architecture and law. Humanities are big, while the arts faculty is both a leavening for the rest of the curriculum and a valuable contribu-

tion to Sydney's cultural life.

Financially, the university continues to grow, but only by means of research grants and donations. Although the federal government's grant (at \$A125.6 million) was unchanged between 1983 and 1984, the administrators complain that the inflation factors used by the Commonwealth Tertiary Education Commission do not adequately compensate for the inflation of academic costs. But the university is now raising more than 21 per cent of its income from other sources, most of them public funds such as the ARGS (see p.194). There is, however, an invaluable \$A4 million income deriving from investments, a pot of gold worth \$A54 million (which should surely be yielding more).

The university typifies those among the larger universities who see their future as one in which they will be increasingly dependent on what they can earn by research and other means. There is, for example, a university company (called Unisearch Ltd) which promotes the role of its academics as industrial consultants, and which also handles joint research contracts and even the exploitation of patents; gross income last year exceeded \$A2.58 million.

Not surprisingly, the search for outside funds runs into the familiar conflict between academic and commercial interests. Professor Raymund Golding, the pro-vice-chancellor for administration, says in the annual report just published that potential industrial partners cavil at the payment of an element of overhead costs and also think they should in some sense "own" the results of successful projects. If what people say about Australian industry's regard for research is true, the learning process will be slow.

The search for outside sources of funds is one of the reasons prompting the creation in the past few years of a series of topic-based interdisciplinary centres with responsibility for teaching and research. Uniquely, it is claimed, there is a centre for law and technology, and others dealing

Popular science

MAKING science public in Australia falls to a variety of agencies, of which the best known and most frequently disappointing is the Australia and New Zealand Association for the Advancement of Science (ANZAAS), whose 1985 meeting will be held next month in Sydney. The Australian Academy of Science does a valuable service, but of a different kind, having become over the past decade a first-rate school textbook publisher.

But Australia's most vivid source of news about science is the Australian Broadcasting Corporation's output of sound radio, and in particular the series of regular programmes built around the weekly programme *Science Show*. The producer, Robyn Williams, says that ABC has given him the chance during the past decade to try a variety of formats. The programmes are both popular and arresting. Especially now that they are complemented by programmes ranging from straight-forward but informal talks to general information about the use of computers, they have contributed to the high awareness of policy on science and technology in Australia. □



Kangaroos thriving but still threatened by drought

DESPITE rumours to the contrary, the kangaroo is alive and well and living in Australia. In recent years, there has been considerable concern, and international pressure, over their commercial killing and some fears that kangaroos could be endangered. Although criticism over hunting methods may still stand, sophisticated aerial surveys show that there are still plenty of kangaroos.

"Kangaroo" is not in itself a scientific term and generally refers to the man-sized members of the Macropods, usually the red kangaroo (the favourite at zoos) and the two grey kangaroos. Among smaller members of the group, the wallabies and rat-kangaroos, there are six endangered species and several others that are rare, although none is commercially hunted.

It is almost two hundred years since the kangaroo was first exhibited in London, at the Lyceum in the Strand; admittance was seemingly expensive at one shilling, but perhaps not for "an Ocular Demonstration [which] will exceed all that words can describe, or pencil delineate ...". And from that time, kangaroos have been killed for meat, for their skins or simply as pests, by gun by poison or by simply rounding them up and clubbing them to death by the hundreds, in the "battue" that occupied sporting Australians in the nineteenth century.

More recently, kangaroos have become a subject of fierce controversy; some would have them totally protected as a unique symbol of Australia, some believe they should be destroyed as pests because they

damage fencing and sheep pastures, while some hold that they should be managed for maximum profit as a renewable resource. International pressure has come from the United States where conservation groups succeeded, in 1974, in having red and grey kangaroos listed as a "threatened species", and the import of kangaroo products banned. The ban was later lifted after Australia was able to demonstrate that kangaroo



resources are properly surveyed and managed. All seemed set for them to be removed from the US list of threatened species but in April 1984, the US Fish and Wildlife service ruled that the severe 1982-83 Australian drought had put the status of kangaroo populations in doubt once more.

The latest evidence suggests, however, that kangaroos may be even more abundant than before European settlers ar-

rived. Thanks to work by Graeme Caughley of the CSIRO Division of Wildlife and Rangelands Research, kangaroo populations can now be accurately estimated from the air. The trick is to fly a series of transects, at a particular height and speed, counting the kangaroos within a 200-m strip marked by streamers trailing from the aeroplane. Not all kangaroos are seen but a set of equations, devised by Caughley is used to correct the observer error according to the type of countryside. When these results are compared with sightings of kangaroos recorded in early explorers' diaries, it does seem that the kangaroos are more abundant than ever before. The probable explanation is that the provision of water-holes for sheep and the reduction of the predatory dingo population have removed constraints on the population.

Commercial taking of kangaroos is now very strictly controlled in all states. Unlicensed hunting is prohibited. But it is unlikely that protest will diminish over the seemingly brutal way in which kangaroos are taken by dazzling them at night with bright lights and finishing them off with clubs. Such practices have gained international notoriety; when the Japanese were under fire last year for whaling, from Australian conservation groups among others, Japanese newspapers ran pictures of Australian kangaroo hunting with a headline raising this awkward question "How can they criticize us?" This is a point the Australians might take.

Alun Anderson

with the ophthalmology of contact lenses (a local speciality), industrial relations and the Japanese economy. To label a group of academics thus may either be a way of simplifying the task of giving outsiders special advice in the form of custom-designed courses or a way of attracting research contracts credibly.

One of the most conspicuous of these centres is also the most unusual, consisting of a collaboration between the university and the Royal Melbourne Institute of Technology. (Sydney and Melbourne are generally linked together only by their rivalry.) The funds for the venture (\$A2 million) were originally provided (in 1982) by the federal government, then founding ten industrially oriented research centres at universities. More recently, the centre has made a deal with the Australian subsidiary of IBM which may not be a direct source of income but which extends the scope of the operations.

The centre's proudest boast is to have produced what are the most efficient solar cells so far to have been made of amorphous silicon (see M. H. Green *et al.* *Appl. Phys. Lett.* **44**, 1163; 1984). The trick is to coat the surface of a piece of silicon (doped with phosphorus by diffusion from the upper surface) with a layer of silicon oxide between 20 and 50 ang-

stroms thick, using a technique developed by M. H. Green over the past decade. The insulating layer inhibits recombination of electrons and the holes from which they have been quarried; electrical contact through the upper, exposed, surface of the cell must be accomplished by a network of metallic fingers evaporated onto the surface. The highest efficiency claimed so far is 19.1 per cent (with an open-circuit current of 0.64 milli-ampere).

Nobody pretends that hand-built cells like this (the standard size is 2 cm square) will be mass-produced so as to meet the world's (and Australia's) need for energy. The next goal, at the centre, is to push up the efficiency to 20 per cent or more. In the process, the centre argues, people will learn to understand what design parameters are needed to build an efficient, and cheap, solar cell. And in any case the laboratory will become even more familiar than at present with the technology of making semiconductor devices; it has ambitions to use thin films of insulators in more conventional transistors.

The facilities at the centre are not very different from those to be found nowadays in microelectronics laboratories elsewhere in the world; the University of Edinburgh in Scotland is, if anything, better equipped. But the joint centre gives

the University of New South Wales (and its Melbourne partner) the chance to acquire and improve basic skills in an important field and, with luck, evens the chance to strike gold one day. The federal government's investment seems to have been worthwhile; next month's budget will show on what scale it is to continue.

For the university as a whole, this is only one of a great many developments that should collectively give the lie to the federal government's suspicion that Australian academic institutions contribute too little to social and economic well-being. Visitors from a land of small universities such as Britain are bound to be impressed both with the financial robustness and the intellectual diversity of a really large university. New South Wales is large enough to be flexible about student entry (more than a quarter of the students are part-timers, a proportion growing towards a third are women) and even to mount special programmes for aborigines and students from other sectors of higher education without formal academic qualifications. It is also large enough not to be ashamed to have an international reputation both in the anatomical description of the mammalian retina (as by Dr Jonathan Stone's group) and the mechanical and optical engineering of contact lenses. □

Adelaide University

The sweet smell of success



THE University of Adelaide's compact campus embodies the whole range of responses to the overall decline of funds for basic research facing Australia's scientific community. Barring a last-minute reversal, the university is switching resources away from science; seven posts are to be taken from the Faculty of Science this year, and transferred into commerce, law, women's studies and engineering. But, at the same time, the university has set up its own venture development enterprise and encouraged new joint ventures with industry, which are now flowering in a number of departments.

Those whose subjects are currently in vogue find little to complain of, and several departments have managed to win various species of the government's new grants. But for those in areas of basic research now out of fashion, or without immediate appeal to industry, the outlook is bleak.

Physics is likely to be one department affected by the shift of jobs to elsewhere in the university, even though the demand for physics graduates is as high as ever, according to annual surveys conducted by the department's head, Professor John Prescott, and despite a recent series of major discoveries by the cosmic ray group. Using the only detector of energetic cosmic-ray showers in the Southern Hemisphere (home-built at a fraction of the cost of others), they have discovered two of the three known sources of ultrahigh energy X-rays — binary X-ray stars in Vela and the Large Magellanic Cloud (see *Nature* 315, 205; 1985).

Good work is also being done by the atmospheric physics group which has installed the first middle-atmosphere radar in the Southern Hemisphere, and by a group interested in the solid-state physics of thermoluminescence dating. They now claim the most sensitive thermoluminescence spectrometer in the world. Not surprisingly, there is a deep sense of frustration that sufficient funds are not available to build on the department's achievements, nor to stop talented workers slipping away to the United States.

Professor Prescott sees Australia's level of spending on science as "appalling" and points out that Australia is seventeenth among the 21 OECD countries in terms of research and development spending as a percentage of gross national product. But that does not mean that all hope has been abandoned: one member of the department, fresh from CERN, is campaigning against all odds for Australia to take up high-energy physics.

A more optimistic outlook on life prevails in the geology department, where Professor David Boyd has just had the go-ahead (and \$A150,000 a year) for a "key" centre, one of the Commonwealth

Tertiary Education Committee's new concepts to promote areas of "special economic and social significance". The key centre is for advanced studies in petroleum geology and geophysics; ten people a year will enter a two-year course and there should be plenty of jobs waiting for them, given the probable acceleration in the pace of oil exploration. Professor Boyd has spent a major part of his own career in industry and the new course will involve the Australian Mineral Foundation, an industry-financed independent institute running advanced workshops. The aim is to mix students with other people in industry; that will, in turn, help industrial funds flow into the department.

In biochemistry, there is also the sweet smell of success. A "centre of excellence" was created in 1982, with a \$A1.2 million grant over three years; this has now been followed by \$A1.6 million for the next three years. The Commonwealth Special Research Centre for Gene Technology, as it is officially known, carries out fundamental research on gene regulation under Julian Wells.

Within that huge area, there are projects on the activation of genes that metabolize drugs in the liver, the isolation of wool keratin genes, plant viruses and viroids, tissue-specific expression of histone genes and the use of recombinant DNA technology to produce growth hormone. Virtually all the projects are planned with some direct application in mind. The drug metabolism studies relate to the human disease porphyria. Studies on wool show cysteine to be a limiting factor for growth, so the ideas of introducing cysteine synthesis genes by embryo microinjection is being explored. Viroid probes can allow

the early detection of economically important plant diseases, and so on.

Indeed, the group has already gone into the commercial business by setting up BRESA — a wholly university-owned biotechnology company. Eight full-time researchers are already at work for the company and have one best-selling product — photobiotin — which allows the simple light-activated coupling of biotin to DNA and RNA probes.

The microbiology department also hit the jackpot last year with the acquisition of a \$A870,000 grant over three years from the government's new National Biotechnology Programme. The grant went to Professor Derrick Rowley for a collaborative project with industry — in the shape of F.H. Faulding and Co. Ltd, a successful local pharmaceutical company, for the development of oral vaccines against enteric diseases. A joint company "Enterovax" has been set up, employing 14 people on research within the microbiology department.

It is not strange that the biologists are now getting the cream of the grants, for the government has to some extent recognized the potential of biotechnology. (Biologists also tend to mutter that physicists had their day of riches in the past; now it is the biologists' turn.) But that said, only seven per cent of applicants for funds from the National Biotechnology Programme received anything at all, an astonishingly high rejection rate which has led to complaints about the programme content, the level of financial support and even the competence of the grant authority. Competition for "key" centre funds was also severe with some very disappointed applicants with excellent schemes. Adelaide University (or at least some parts of it) must therefore count itself among the blessed.

Alun Anderson

Wildlife research

Vermin are out — fauna in



AUSTRALIA'S interior contains a veritable menagerie of the descendants of settlers' domestic animals. Besides the rabbits, there are wild donkeys, horses, goats and pigs; enough camels to provide for a roaring trade in their export to Saudi Arabia; and, in the tropical wetlands east of Darwin, around a half million water buffalo. It is not surprising then, that the principal wildlife research organization, CSIRO's Division of Wildlife and Rangelands Research, carries out an inextricable mix of research on pests and on wildlife behaviour, ecology physiology and conservation.

The division is itself a recent marriage; the Wildlife and Rangeland Divisions were formerly separate, but it was felt that in these days of ecosystem studies, there was no further virtue in keeping plants and animals apart. The division provides for

long-term fundamental studies; shorter-term and day-to-day management problems of conservation areas remain in the hands of the park and wildlife services of the states.

Australia is well covered with parks of one kind or another. Of the total land area, 3.7 per cent (28 million hectares) is specifically reserved for nature conservation. State by state, the proportion of land set aside for conservation varies greatly, reaching 25 per cent in the tiny Australian Capital Territory and 14 per cent in Tasmania, but is less than 1 per cent in the sparsely populated Northern Territory. The Great Barrier Reef is protected by a specially constituted Marine Park Authority.

The division responds to requests for fundamental research from the various states, and also takes on work funded by



Laying down the law on wildlife protection

TRAVELLERS beware! On your stop-over in South-East Asia on the way to Australia, do not be tempted by souvenirs of ivory, exotic butterflies, bird feathers or shells. If you succumb, a gaol sentence may await you.

From May 1984, Australia has had what are probably the world's strictest laws regulating the export and import of wildlife and plants. The native fauna of the nearby developing countries could be ruined by tourists prepared to pay fabulous sums for exotic souvenirs. But the new act provides for up to five years' imprisonment and a \$A200,000 fine for those so tempted.

Indeed, the new laws were considered so strict that \$A0.25 million was spent publicising them before they came into effect — and two gold medals for the best advertising campaign of the year were won in the process. Australia's action will undoubtedly help stop regional trade in endangered species; if Japan could be induced to take similar tough action, real progress might be made.

Responsibility for administering the Wildlife Protection (Regulation of Exports and Imports) Act lies with the Australian National Parks and Wildlife Service

(ANPWS), set up in 1975 as the principal adviser to the Commonwealth government on national nature conservation and wildlife policy. This duty includes the job of representing the government at CONCOM, the council of nature conservation ministers drawn from all the states, and on international bodies such as the International Whaling Commission.

ANPWS also has responsibility for some 5,000 galleries of paintings — all of them out of doors, on rock, and some 40,000 years old. That makes them by far the oldest paintings in the world, and according to Professor J.D. Ovington, the service's director, they are also "among the most beautiful".

The paintings are all in Kakadu National Park in the Northern Territories which, along with Uluru Park (Ayers Rock and Mount Olga) and Christmas, Norfolk and other island territories, are administered directly by ANPWS.

The aim is to set standards that state authorities will emulate (although some may not like to hear that). Certainly, considerable praise has been won for the Kakadu Park's involvement with the local aboriginal community; older people have

been appointed as cultural advisers and many younger people have been trained as park rangers.

ANPWS also has sufficient funds to assist directly conservation programmes of national significance. Needs are diverse and last year the service helped more than thirty projects, ranging from a national plan for dealing with mass strandings of whales to the use of airborne scanners to map vegetation. Odd situations arise; when Vietnamese refugees with quite different eating habits arrived in the country in large numbers, there emerged a risk that they might decimate some marine animals that Australians had not thought of as food.

On a more mundane level, ANPWS produces a staggering output of educational literature — part of which includes the postcards shown above. Indeed, if the number of posters and brochures on Australia's unique wildlife and the masses of endangered species leaflets and stickers are anything to go by, exemplified by the postcards shown above, Australia is well on the way to becoming the world's most conservation-conscious nation.

Alun Anderson

the various agricultural product boards and the like. Some 12 per cent of its \$A8 million budget comes from such outside sources.

Thus the Wheat Board is now supporting research on one of Australia's more bizarre problems: the so-called "mouse plagues" that erupt at four to twenty year intervals in the wheat belt of south-eastern and eastern Australia. Between plagues, mice are rare and of little economic account. When a plague gets under way, they appear locally in their millions producing, as one surrounded householder put it, "a moving carpet of mice, in some places three and four deep... when you walk you tread on them and they run up your legs".

The mice eat everything they can; crops, seeds and the wiring and upholstery out of cars, tractors and harvesters. They penetrate through wooden walls, devour furniture and clog up irrigation pumps. In a 1979 plague in Victoria, \$A15–20 million

was counted, but that is probably an underestimate; a considerably less severe plague in 1984 caused \$A30 million worth of damage.

The problem is perplexing, for the plagues are local and sporadic. Work by Trevor Redhead at the division indicates that the essential trigger for a plague is above average rainfall in autumn which lengthens the time for which the mice can reproduce in winter. But the mechanisms by which the population build-up can reach "plague" levels is far from being understood.

The division's work is not all plagues and pests, however. Basic work by L. Braithwaite on tree-dwelling marsupials, the possums and gliders, suggests that changes in logging practice could reduce the impact of commercial forestry on their populations. The eucalypt forests in which the animals live are commercially exploited, and conventional wisdom has it that harm to wildlife can be avoided by

cutting alternate plots of land in a long-term cycle. But the real situation is not so simple: the marsupials prove to have very marked preferences for certain trees, preferring the so-called "peppermint" eucalypts. This turned out to be because the leaves of the peppermint eucalypt contain high levels of nutrients and so can provide more food for the leaf-eating marsupials and more insects for the insect-eating marsupials.

The Forest Commission has taken note of the patchy distribution of fauna, but a problem remains. The nutrient-rich trees grow preferentially on nutrient-rich soil, much of which, because of its economic potential as farmland, is in private hands. So as is common in wildlife research, the future of the possum and glider marsupials must be passed from scientists to politicians. A way to resolve a potential clash of interests between the needs for faunal conservation and agricultural production must be found.

Alun Anderson

Nuclear energy

The slumbering giant



WHATEVER can have happened to nuclear energy in Australia? A quarter of a century ago, the Australian Atomic Energy Commission, like all its counterparts, was busily designing a nuclear reactor, urging on the exploration for uranium (for which it was the government's sole agent) and even dropping heavy hints that peaceful nuclear explosions might be used to create harbours in the otherwise inhospitable west coast. Australia still contains the world's largest reserve of uranium ore, more than a quarter of the world's total, but otherwise there is a deathly silence.

What has changed? The political temper of Australia, which is not the same as party politics. Australia has acquired in the past decade a detachment from the world of powerful alliances. The close alliance with Britain, now seen as subordinate membership of the old Empire, has long since faded (and may entirely disappear when Judge McLelland's report on British nuclear testing in Australia appears later in the year). The brief flirtation with the United States in the 1960s (Australia was the only other country to send a force to Vietnam, and its then prime minister invented the phrase "All the way with LBJ!") has also withered, as Australia looks more closely around the Pacific rim.

Just over a decade ago, people in Australian television talk-shows were forever arguing about the wisdom of exporting raw materials to economic rivals; why not add the value here, in Australia? Better leave the minerals in the ground, some said. Since then, the long-standing Australian concern that others should not make weapons from its uranium has been strengthened, not least by the French nuclear tests in the Pacific. All political parties include a powerful section of opinion that uranium should be left in the ground. Mr Hawke's Labor Party is riven on uranium policy.

The dilemma for the present government is that the political risk of governing the opponents of uranium mining are offset by the political risks there would be in losing all those jobs, not to mention an export trade worth upwards of \$A2,500

million a year. (Coal exports, now running at \$A4,000 million, are worth even more.) That, no doubt, is why, in November 1983, Mr Hawke asked the Australian Science and Technology Council (ASTEC), and its chairman personally, to study "Australia's role in the nuclear fuel cycle".

ASTEC's response, published last May, failed to resolve the dilemma. The committee endorsed the traditional Australian view that uranium suppliers should do everything they can, by imposing conditions on the uses to which their uranium may be put, to inhibit the proliferation of nuclear weapons. But the council's report went on to say that exports should not be limited "as a matter of principle" but instead should be permitted under stringent conditions. And after endorsing government policy on the control of nuclear weapons (suggesting, in passing, that Australian bilateral agreements should be published as models for other suppliers) went on to argue for Australian participation in "stages of the fuel cycle other than mining and milling" if that will help strengthen the "non-proliferation regime".

Last summer's Labor Party caucus on uranium policy was apparently contentious. This may have been when Mr Barry Jones, Minister for Science, blotted his copybook by opposing the ASTEC recommendations (see p. 188). In the event, Mr Hawke was forced to announce the rejection of the two contentious proposals: mining would be allowed at only a third mine in South Australia if that proved economic, Australia would not participate in other stages of the fuel cycle and this decision will not be reconsidered at least until the end of 1987 (by which time there will have had to be another general election).

Opinions remain divided. The case for doing something to the uranium, enriching it in uranium-235, for example, had seemed strong enough until last year for the Atomic Energy Commission to be hard at work on the centrifuge enrichment process; now that programme has been converted into what is described by Dr D. G. Walker, the director of the commission's laboratory at Lucas Heights, north

of Sydney, as a study of the applications of anti-proliferation measures to the centrifuge process.

Against the background of the Hawke announcement, it is a little strange to find that the Lucas Heights establishment has shrunk since its peak a decade ago. There are around 1,000 employees; most of the fall from 1,300 a decade ago is explained by the transfer to CSIRO of the previous programme of research on unconventional energy sources. Yet nobody seems worried about his job.

Part of the explanation is Synroc, the name for the project that has grown from the advocacy of Dr A.E. Ringwood, from the Australian National University, that would incorporate highly-active waste in synthetic rock. Ringwood's process, not yet proven with radioactive materials of the kind for which its use is planned, has given Lucas Heights a new lease of life. The commission has signed agreements on collaboration with the United Kingdom and Japan.

For the rest, the establishment has three functions. It is a major source of short-lived radioactive isotopes not merely for Australasia but occasionally for more distant customers. (West German non-destructive testers used to buy substantial amounts of ytterbium-169.) More recently, the establishment has developed a neat way of extracting technetium-99 from the fission product molybdenum-99 in such a way that physicians can extract small amounts of this useful tracer when needed simply by eluting it from alumina.

Lucas Heights is also a general-purpose research establishment (in agriculture, hydrology and nuclear physics) and a teaching resource (with a 3-MeV Van de Graaff machine used chiefly for fission studies). What would be said of a uranium-exporting country that had no means of telling what fission is?

The third function is advisory. Simply because of the government's interest in proliferation, a small army of experts in the field is a necessity. This need is unlikely to vanish. And in any case, Lucas Heights is a nuclear development establishment in waiting, the kind of place that would be needed if a change of policy came along.

The critics of Mr Hawke's uranium policy are of two kinds, those who would leave the material in the ground and those who hold that it is too restrictive. The next dispute is likely to blow up when the commission asks for a replacement for its HILAR reactor, modelled on a British reactor built in the 1950s. Dr M.H. Brennan, the Sydney physicist who is also the commission's chairman, thinks the request cannot be long delayed. Others go much further, pointing out that there are already many remote parts of Australia where a cheap and small-scale source of nuclear electricity would be a boon. And the present economic upheaval may make many people regret the loss of all that value added. □

The leading producers of uranium (non-communist countries)

Country	No. of plants	Capacity (tonnes per yr)	Estimated reserves (tonnes)
United States	33	13,750	131,300
Canada	5	8,540	176,000
S. Africa	15	5,780	191,000
Niger	2	4,450	144,000
Namibia	1	4,250	119,000
Australia	2	3,950	474,000
France	6	3,330	56,200
Gabon	1	1,500	18,700

Figures for uranium production are as at June 1983, from estimates made by the Australian Atomic Energy Commission. Reserves are "reasonably assured resources" for 1983 based on a cost range to US\$80 per kg. Values for other countries other than Australia from OECD. No estimates are available for the Soviet Union, Eastern Europe and China.

Defence research

Marshalling scant resources



THE largest research and development complex in the Southern Hemisphere sits some 24km outside Adelaide. It has a staff of 2,660, five square kilometres of grounds, 1,300 buildings and many, many kilometres of high wire fence. It is the main research centre for the Defence Science and Technology Organization (DSTO) and appropriately enough, for those familiar with British military manoeuvres, is just beyond a town called Salisbury Plain.

Besides the Salisbury centre, there are some eight other defence research laboratories around the country, with a total staff of about 4,400, but with fewer research scientists than CSIRO. Annual expenditure runs at \$A140 million or 2.7 per cent of the defence outlay, which is itself 2.7 per cent of gross national product. That is just one-twentieth of the British defence research and development budget, a statistic which explains much of DSTO's character; it seeks to cover the whole range of technical problems even with a very much smaller capability.

Inevitably, most defence equipment is bought abroad rather than developed in Australia and a great deal of DSTO's energy goes into maintaining the ability to give advice on equipment needs as well as adapting foreign equipment to the very different operating conditions of the Southern Hemisphere. Indeed, the spread of knowledge required is such that, occasionally, all the expertise in one area resides in just one individual — to whom it is earnestly hoped that nothing untoward happens.

A little research capacity is, however, left over for the development of quite new concepts. Past successes include Malkara, the world's first wire-guided antitank missile, bought by the British during the Suez War of 1957 when they suddenly realized that they had sold the then impregnable Centurion tanks to the other side. And, after that, there was Ikara, a missile-borne homing torpedo still widely used.

Present public fantasies about DSTO's activities include, if the popular press is a guide, a "death ray" or, more precisely, an electromagnetic rail gun which the United States is said to be keen to see as part of its "star wars" (Strategic Defense Initiative, SDI) inventory. Rail guns fire metal slugs at very high speeds by electromagnetic pulses: one Australian scientist has done some neat work (in the United States) on the power pulses that provide that drive. But there is no major development programme under way and the possibility of involvement with the SDI programme is still a subject of fierce political debate.

There are, however, two big defence projects of worldwide significance. In one, Australia is up with the leaders. The Jindalee over-the-horizon radar system

based at Alice Springs can illuminate an over-the-horizon target by bouncing a high-frequency radar signal off a refracting layer in the ionosphere; the signal will return by the same route, and is picked up by a 2.6-km-long linear array. Clever signal processing is then needed to pick up the target against noise returned from hundreds of square kilometres of background. Essentially, flying objects are detected by their Doppler-shifted signals; ships can be picked up because they are stationary against a background scatter Doppler-shifted by moving waves.

The second project, Winnin, was aimed at building a decoy for Exocet-type anti-ship missiles long before their lethal efficiency was demonstrated in the recent Falklands War. The decoy is now at the "proven concept" stage, awaiting a foreign partner for further development, and was exhibited at the Paris Air Show in May. It consists of a remarkable rocket which can hover, nose up, at any desired height above the ground and which, by a slight tilt, can move from side to side, horizontally. The advantage of using a rocket as a decoy is that it can be launched in just seconds — after an Exocet has been detected, a maximum of two or three minutes are left before impact. The rocket can then hover and move very slowly sideways, just as though it were a ship, so that

the Exocet radar cannot readily tell it is a decoy. To complete the deception, the decoy would carry a sophisticated piece of electronics to amplify and return the missile's radar signal to lure it away from the smaller signal given by the intended target, the ship.

A host of other devices have been developed by the various branches of DSTO. These include the Karinga cluster bomb, the BARRA sonar buoy, and the LADS laser airborne depth sounder which can map 50 km² of ocean an hour with a scanning green laser beam that penetrates to the sea floor. Not everything is designed for purely military use: there is also an airborne neonatal intensive care unit for "flying doctors" and a cheap and tiny parachute which allows water bottles to be dropped from the air without bursting.

Financial pressures on DSTO seem not to have been as great as those affecting CSIRO and the universities. Australia's lack of a large-scale defence industry virtually assures that most research and development must remain within a large government organization, although close links are maintained with such defence industries as exist. Support is given to keep engineering expertise in industry from dispersing between contracts. For the future, the chief problem will be whether the thin skin of experts can be stretched to cover the ever-expanding technology of defence.

Alun Anderson

Australian Institute of Marine Science

Aiming to count starfish



THE Australian Institute of Marine Science (AIMS) at Townsville must be about the only research laboratory in Australia to be reaping some benefit from increasing unemployment. Thanks to the Commonwealth Community Employment Programme, \$A1 million has been found to give 55 people work on a one-year study of the distribution of the Crown-of-Thorns starfish on the Great Barrier Reef.

Other than this welcome inflow of money from a department not normally associated with science funding, AIMS faces much the same problems as other institutes; its budget, currently \$A7.3 million a year, is not rising, but inflation is driving overheads ever upwards. So far this seems not to have dampened the enthusiasm of AIMS's staff who, to the institute's great good fortune, are largely in their thirties and perhaps at the most creative part of their careers.

The institute too is young; it was set up 11 years ago under an act of parliament which gave it responsibility for research in the entire field of marine science and has been handsomely provided with facilities, including three research vessels, the largest of them 24.4 metres long. Uni-

versity researchers speak enviously of "luxurious blue-carpeted halls" (which really do exist), but as the institute is located 50 km out of Townsville, at the end of a 20 km private road, used mainly by hundreds of wallabies, the opportunities for sack and pillage are slight.

Despite the very wide brief AIMS was given, it is not actually able to send scientists very far afield. Research remains firmly linked to the Great Barrier Reef and to the tropical coastline of mangrove forests further to the north.

Current worries are that the reef may once again suffer a massive increase in numbers of the Crown-of-Thorns starfish, similar to that in the late 1960s and early 1970s. The starfish eat the hard-bodied corals, and the result of an outbreak, when starfish can be seen on a reef by their hundreds of thousands, is a change from 45 per cent coral cover to just two or three per cent, with accompanying catastrophic changes in the rest of the reef community.

That the starfish are once again on the increase is suggested by reef surveys and by questionnaires returned to the Great Barrier Reef Marine Park Authority by vacationing skin divers. For AIMS, however, what is most certain about the

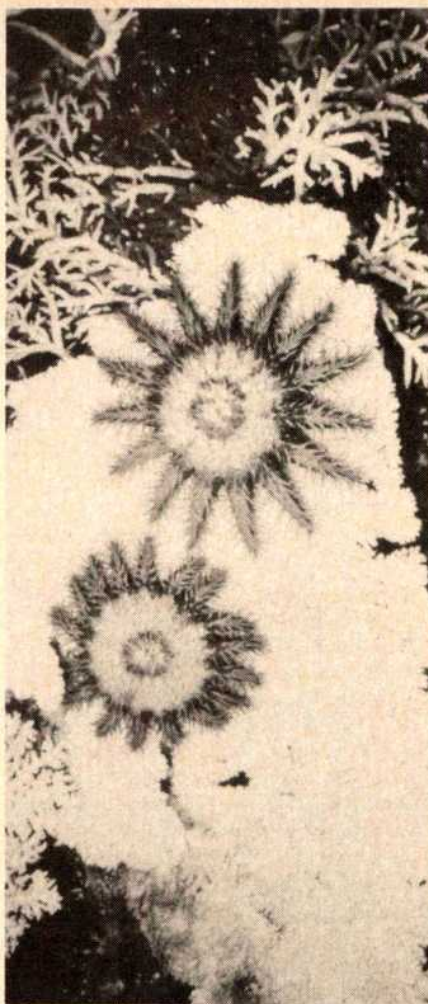
outbreak is the vast uncertainty. Nobody knows the true extent, or the dynamics, of the changes in the starfish population, hence the Department of Employment programme, funded to provide a one-year "snapshot" of the coral-starfish system. Two hundred and twenty eight types scattered along the whole length of the reef are to be sampled.

So far at least it does not seem as if the reef is heading for destruction. Areas known to have been decimated 10–15 years ago appear to have recovered their coral cover, although it is not known if the reef is quite the same as it was before. It is entirely possible that outbreaks of the starfish are "natural" and have been continuing for vast spans of time. Cyclic changes in starfish abundance with complex dynamics can be driven simply by predator/prey interactions, as recent mathematical modelling at AIMS shows. On the other hand, cycles may have been triggered by some external factor. Either way, the possibilities for control seem remote. In Japan, an attempt was made to prevent a Crown-of-Thorns outbreak by removing an astonishing ten million starfish from reefs in the Okinawa region, but without success.

Research on the Crown-of-Thorns starfish is but one (temporarily well funded) part of AIM's activities. Elsewhere, diversity is the name of the game, and AIM's activities well illustrate how basic research can confound the ill-informed among politicians and have consequences of economic and perhaps profound social value.

Why, for example, are coral reefs free from bryozoans, tunicates and so on that are found encrusting nearby piers and pilings? The answer proves very simple. In the clear waters of the reef, the levels of ultraviolet light are simply too high to permit their growth — put a sheet of ultraviolet absorbing Perspex on the surface and they will grow in abundance. (Those who hold the popular misconception that ultraviolet light cannot penetrate far in water should read N.G. Jerlov's 1950 *Nature* paper which shows quite the contrary.) The question then becomes one of why corals are not similarly affected by ultraviolet radiation. The answer proves to be that they contain a natural "sunscreen", a UVB blocking agent. This has now been isolated and seems set to form the basis of a commercial sunscreen, or even a marine plastics and paints additive. Results of the research are being held back from publication until formal patents have been filed; a contract with a chemical company to develop the agent has already been signed. Preliminary announcements were feted by Barry Jones, Minister for Science.

Results with broad economic consequences could also stem from a second study (published in part by *Nature* last August) which began when it was found, contrary to received wisdom, that fluorescent



The Crown-of-Thorns starfish again threatens the Great Barrier Reef.

bands appear in sections cut from long-lived *Porites* corals. The bands, in corals growing on the shore side of the reef, turn out to be caused by fulvic acid leached from soil by water; they thus provide a record of nearby river output, and so of rainfall, which can be dated to within a few weeks by X-ray examination of the annual growth rings of the coral. As massive corals may be many centuries old, they thus contain a long-term record of floods, droughts and river flow — exactly the sort of information that saves huge sums of money in the planning of dams and flood control systems.

As well as showing annual cycles, the bandings record much longer-term patterns of atmospheric pressure. The record in the corals has the potential to push knowledge of Australian meteorological conditions back several centuries. What makes this so important is that the region is critical for the Southern Oscillation, the unpredictable shift in weather conditions which caused both the recent Australian drought and the El Niño phenomenon with its profound economic consequences for South American fisheries and indeed, the whole of the South Pacific region. It is too soon to be sure, but the corals could be the first long-term record on which climate modellers can work.

Alun Anderson

Antarctica



Glaciology still on ice

SEEN from Australia, Antarctica is not a remote land at the uttermost end of the Earth but, rather, the nearest southern neighbour, closer to Sydney than either Singapore or Jakarta. Almost half of the continent — some eight million square kilometres — is claimed by Australia. And, uniquely, Australia's claim is disputed by no other nation.

For the time being territorial claims may matter rather less than does the smooth working of the Antarctic Treaty, which has kept the area demilitarized and unexploited and has, so far, provided a model of international scientific cooperation.

Keeping things this way is very much in the Australian interest. Militarization would expose Australia's southern flank, and changes in the environment could affect Australia's weather. Politicians have thus always stressed the importance of maintaining a visible presence there.

For Professor David Caro, vice-chancellor of Melbourne University, and for seven years chairman of the Antarctic Research Policy Advisory Committee (ARPAC), however, Antarctic visibility should mean only one thing — scientific research. But as things are, it seems there is just enough money to get men there but not enough for them to do very much. As he puts it, there has been a "credibility gap between what territory Australia claimed in the Antarctic and what it did there".

Professor Caro is about to end his chairmanship after seven years of what he calls "butting against a brick wall". But there have been some notable expenditures on the Antarctic effort in that period even though they have often been controversial. Sixty million dollars is being invested over ten years in rebuilding the three stations at Mawson, Casey and Davis — to the accompaniment of criticisms that the new designs are too expensive. As Minister of Science Barry Jones put it, "we have the best buildings in Antarctica, but we do not lead in much else". Building methods are now being reviewed and attempts made to cut costs by modular construction. More worrying is that the massive high-quality heavyweight buildings under construction will tie researchers to those particular locations for ever. A cheaper, less permanent solution might well have been preferable.

Construction should, in any case, speed up now that a five-year charter has been taken on a Norwegian supply ship *Icebird*. The new ship will also help one area where research has expanded and no complaints are heard — marine biology. Also in the "not too bad" category are cosmic-ray and meteorological research, which are continuing because they can be carried out

close to the bases. But it is a different story for glaciology and geology — areas in which Australia was once strong. The glaciology has petered out for lack of the tractors or helicopters to make this work possible.

A further bone of contention is that air strips are not maintained at the bases, so that senior scientists whose time is too limited to allow for the six-week voyage cannot fly to Antarctica. This is ironic as the Soviet Union is able to fly to its bases right in the middle of Australia's claim.

On the government side, the Antarctic Division of the Department of Science points out that overall spending has risen, although it has tended to be true that the government has accepted only the cheapest recommendations from ARPAC. But the government is proud of the political initiatives it has taken in Antarctica. The Convention on the Conservation of Antarctic Marine Living Resources came into force in 1982 largely through Australian efforts and is administered from Hobart. The convention is progressive in that it covers all living organisms in the Antarctic ecosystem. Management guidelines to ensure the ecosystem's stability are worked out by the scientific commission of the convention.

Australia is now active in promoting a similar convention on mineral resources; meetings of the treaty members have been taking place every six months. Progress has been steady, but there are potential problems. The parties to the Antarctic

Treaty consist of the seven original "claimant states", nine "full consultative" parties who have paid the heavy entry fee by setting up research facilities in the Antarctic and a further 16 "non-consultative signatories" who enjoy observer status at treaty meetings. Now the whole system has recently come under attack, particularly from Malaysia, which demands that Antarctica should become the "common heritage of mankind" rather than be run by nations rich enough to set up bases there. But conservation groups are concerned that a deal conducted behind the closed doors of the treaty meetings will permit some form of mineral exploitation, with concomitant risk to the environment; the fact that rich but resource-poor countries such as West Germany and Japan are increasing their Antarctic activity is seen as particularly suspicious.

It is in this climate that ARPAC has, time and time again, pointed out that "if Australia is to have an effective influence in mineral negotiations ... and make a significant contribution to the protection of Antarctica from any adverse effects of mineral exploitation", then it should fund research into the geology and related environmental factors of the Antarctic. So far these pleas have fallen on deaf ears. But there is now talk of setting up a new base, on the sliver of the Australian claim to the west of the Ross Sea, to complement the three sited further to the west.

Alun Anderson

Universities

Accessible but ailing science



THREE types of scientist can be distinguished in the New Zealand university research community: those who are furious about their current situation, those who profess philosophical resignation and those who are thinking of moving somewhere else. A fourth, and much rarer, species has found contentment in some external source of funds. Such was the impression gained when a group of scientists aired their frustrations at a small meeting at the Victoria University of Wellington. The essential problem is that there is so little money available that those who wish to do experimental science and to publish in international journals feel that the odds are against them.

One person who has decided to move elsewhere is Professor Ian Axford, vice-chancellor of the university. He is to return to a Max Planck Institute and feels deeply pessimistic about New Zealand's situation, particularly for young scientists, whom he describes as "totally demoralized". Conditions are indeed difficult and even British scientists would find them hard to bear. Funds available per student are 30 per cent less than in Britain and student/staff ratios are 50 per cent higher — which means enormous teaching loads.

But the main complaint is the virtual impossibility of buying a large piece of equipment. And as exchange rates fall, things are becoming critical for books and journals too. Wellington University's library is spending \$NZ1.5 million a year on its library out of the \$NZ25–30 million slice of the running grant it gets from the University Grants Committee. The fall in the real value of that money means fewer journals, and fewer delivered by airmail, which puts the rest of the world even further away.

This points to the second big problem — travel opportunities. It is best not to ask a group of New Zealand academics if they feel they have ample opportunities to visit world research centres. Sabbaticals are actually quite generous; leave can be taken just once every three years, and an air fare is provided as far as Perth (in Western Australia) and then an allowance (or pittance) of \$NZ20 a day. The only hope for improvement lies in increasing the number of international conferences being held in Australia. Faced with lack of funds and isolation, it is small wonder that some prefer the philosophic view and look at the advantages in being away from "the international hubbub", the opportunity to



Christchurch University's new campus lies among woodland on the banks of a tributary of the Avon.

sit back and "be highly selective about what you do". But this seems too much like making a virtue of necessity to appeal to the majority.

A dissenting view may also be heard from those who see the role of the universities somewhat differently. Dick Earle, professor of biotechnology at Massey University, says his main objective has been to establish a pool of well-trained graduates who could take biotechnology into industry, and in that he claims to be succeeding. He works closely with the frozen meat industry, but his research is not dull. He is working on the computer modelling of freezing objects with complicated shapes — the problem is one of phase change at a complex moving boundary, for which there is no analytic solution.

Many university researchers have made formal links with industry or the Department of Scientific and Industrial Research. Ten years ago, there was almost no contract work in universities: now engineers most noticeably, but also chemists and physicists, have taken on industrial work, chiefly because of financial pressure.

Underlying all the individual complaints made about the state of the universities is a sense of neglect. It is not just that there are problems, but that university scientists have, as one of them said, "no-one to wing to". Nobody really cares about them; nobody represents them at a level of government close to where decisions are made. Government, anyway, has often been singularly proud of its anti-intellectualism and has enjoyed making Aunt Sallies out of academics. But there are hopes that this will change as a body something like Britain's Science and Engineering Research Council may well emerge in the next year or so.

Within the present framework, there are varied opinions on the prospects for reform. One possibility would be to stop the too-even distribution of funds. A stiff dose of competitive peer review to replace the cosy "three wise men" might concentrate funds where they are needed and so concentrate the minds of those with real ability.

Professor Axford takes a more radical

Paying for the universities

RESPONSIBILITY for university finances rests with the University Grants Committee (UGC), whose main job is to assess the funds required to run the universities and to ensure that they are equitably distributed. Needs are assessed quinquennially and now amount to \$NZ200 million a year.

Division among the universities is determined by a formula related to student numbers, and is given as a block grant which can be spent as the university sees fit. Eighty per cent inevitably goes in salaries, much of the remainder on maintenance. What is left can be distributed to meet research costs. The system guarantees that all researchers are equally provided for in basic supplies, but it cannot provide large sums or expensive pieces of equipment.

To provide for equipment (that is, anything that an individual university cannot afford from its block grant, generally reckoned to be about \$NZ5,000) a separate UGC fund is available. But currently that stands at just \$NZ3 million a year, having just been hiked up from \$NZ1.8 million at the start of this quinquennium. Another \$NZ3 million a year is available for the replacement of equipment, and enough funds are provided to support 80 doctoral students a year. With possible claims on

the equipment money are some 623 "basic" researchers, 600 "applied" university scientists (including engineering, forestry and the like) as well as 314 doctors, dentists and vets.

The money is doled out not by independent peer review but "three wise men", who interview everyone who applies for a grant. Grant applications run at about three times the value of available funds. Mr David Hall, director of UGC, says, "we never really fund anything totally". It is expected that universities will always find some money elsewhere, by collaborating with the DSIR or otherwise.

New Zealand's seven universities are:

- University of Auckland
- University of Waikato (at Hamilton)
- Massey University (at Palmerston North)
- Victoria University of Wellington
- University of Canterbury (at Christchurch)
- University College of Agriculture (at Lincoln, near Christchurch)
- University of Otago (at Dunedin)

Auckland is by far the largest university with nearly 13,000 students. Canterbury, Otago, Wellington and Massey have 6,000–7,500 students each, Waikato slightly fewer than 4,000 and Lincoln 1,750. □

view. He believes that "basic science is withering" and, partly because New Zealand universities are too small, that success may "not be achievable in the existing framework". He proposes a single Institute of Advanced Studies responsible for "all postgraduate teaching and staffed with groups of more than critical size for strength and survival". Those working elsewhere, at what would become the equivalents of university colleges, would have to be given free time for study visits.

Despite all the problems, there remains one aspect of New Zealand universities

that greatly impresses outsiders: entrance to the universities is "open" for those over 21. At Wellington, 40 per cent of the students are over 25: it is not necessary to study full-time and grants are available to those who have not been to university before. Indeed, Wellington's oldest student is 82 and now doing a doctorate in classics after passing both BA and MA courses. As Professor Axford put it, "it is a very civilizing thing, which improves the whole tone of the community and stops intellectual fossilization". More of that might be welcome elsewhere. **Alun Anderson**



Buildings from the last century, now housing university departments, mingle with modern buildings on Auckland's campus. In the background, the science block.

DSIR



Industrial disease

THE Department of Scientific and Industrial Research (DSIR) is the giant among New Zealand's research organizations: it consumes 45 per cent of the government's science budget and contains almost a thousand scientists, backed up by over a thousand technicians and support personnel, working in twenty-four divisions.

Virtually every area of science is covered — from oceanography and ecology to industrial processing and plant diseases — in what must be one of the world's last comprehensive government research bodies. Elsewhere, similar bodies have grown and fragmented; in New Zealand, the small size of the population and available resources has continued to make it essential to concentrate researchers in a few organizations so that there is some chance for them to reach a "critical mass" for efficient research.

Now, like equivalent organizations elsewhere, DSIR is under pressure to move closer to industry and to recover an ever-increasing part of its costs from the end-user. In 1985–86 its budget was to have been \$NZ97 million, and it will actually be \$NZ95 million; in the succeeding years it will fall to \$NZ90 million and then \$NZ82 million.

The director-general of DSIR, Jim Ellis, interviewed before these figures were announced but clearly having a good idea of what was coming, professed no pessimism about the future, however. At present, \$NZ13 million a year is raised from services, giving the DSIR a true budget of \$NZ110 million, and he believes that there are still hitherto sacrosanct areas, particularly in agriculture, where it would be fair to expect a bigger return from services.

Others within the DSIR divisions (see below) are very much more troubled about the effect the government cut-backs may have. Some too are much concerned about the increasing pressure to do research with short-term benefits. Jim Ellis himself characterizes a good half of DSIR's work as "very mundane, taking in the washing for a lot of government departments that don't have scientific facilities". That even extends to analysing alcohol levels in blood samples for the police. The rest is "long-term strategic research" intended to have application at some time in the future, plus cooperating on joint projects with industry.

But visits to DSIR research laboratories suggest it is not quite as simple as that. Anywhere you go will reveal lots of long-term, basic research going on, much of it no different from what would be found in a university, but rather well concealed behind the camouflage of an applied project. The strange thing is that it often

DSIR consists of three main groups, each containing numerous divisions:

Primary production group

Applied Biochemistry
Crop Research Division
Entomology Division
Grasslands Division
Horticulture and Processing Division
Plant Diseases Division
Plant Physiology Division

Resources group

Botany Division
Ecology Division
New Zealand Geological Survey
Geophysics Division
New Zealand Oceanographic Institute
Science Information Division
Soil Bureau

Industrial Group

Applied Mathematics Division
Auckland Industrial Development Division
Chemistry Division
Christchurch Industrial Development Division
Industrial Processing Division
Institute of Nuclear Science
Physics and Engineering Laboratory
Wheat Research Institute
Information Technology Division

Separately administered are:

Antarctic Division
Geothermal Research Centre, Wairakei

looks as though it is the basic work that will, in the long-term, be much the more valuable.

Jim Ellis is also quite aware that lurking within DSIR are all sorts of odd characters with ideas of their own — and he is very proud of some of them. One story he tells is of the world's first natural gas-to-gasoline conversion plant, being built in New Zealand. A by-product of the xelolite conversion is a polycyclic aromatic called druedurene — which is a real nuisance because it crystallizes in carburettors and blocks them up. But now, thanks to one eccentric's work, it has been discovered

that druedurene can be turned into a high-impact strength polyimide plastic.

New Zealanders clearly value their individuality and around the labs one is often impressed by a neat solution someone has found to a problem — but usually to a rather small problem. In the DSIR's Physics and Engineering Laboratory (PEL), for example, a very neat little laser interferometer machine had been built that can automatically calibrate surveyors' staves at great speed. Much imaginative thought has gone into the work, but the gains might be greater if the same people were to be let loose on something bigger.

Another oddity perceived on a whistle-stop tour is that people are often quite unaware of the activities of other divisions — even when they are concerned with related disciplines. This is particularly curious in a country that is almost small enough for everyone to know one another and perhaps a result of the natural tendency of New Zealanders to be happier left to go their own way. But one gets the impression that for any given problem it could be quite hard to find just the right sort of person to help you. DSIR is certainly aware of this and with the Science Information Profile Systems (SIPS) being installed on the DSIR computer network is trying to remedy it. It will be possible to call up information on any number of staff or to find relevant personnel through keyword entries.

These information activities accompany a recent surge of activity in publishing glossy information pamphlets and advisory manuals for industry. Maybe before long they will be forced to stop telling the famous joke about DSIR: "What's the best way to run a small business? Buy a big one and ask the DSIR for advice".

Alun Anderson

Forestry's fears

A MASSIVE transformation lies ahead for New Zealand's forest industry. At its heart is *Pinus radiatus*, introduced a century ago from California, where it is known as Monterey pine, but which grows phenomenally well in New Zealand. Planting is now at such a pace that, early next century, exports of forest products will probably have increased seven-fold, making forestry a major economic force.

The difficulties involved in this transformation occupy the minds of the 300 scientists and technicians of the Forest Research Institute (FRI), the research arm of the Forest Service. The switch to radiata pine is not the only change. Logging of indigenous forest has been virtually halted and the seven million hectares remaining must now be managed for recreation or as "protection" forest to prevent soil erosion or flooding. Just what the effects may be in a country prone to floods and landslides is one of FRI's problems.

The radiata pine is the most immediate challenge. Harvesting will be done in quite new kinds of areas, while the wood is of an unfamiliar type. Silvicultural regimes, which have a profound effect on wood quality, will have to be worked out by computer modelling of tree growth. New mechanical winches will be needed, while there are unknown dangers from pests. The quality of the wood means that new methods of cutting and processing must be developed. And so on.

FRI is facing these new challenges with enthusiasm but worries that the long-term research required in forestry will be hit by the demands of the government for short-term returns, despite the potential of the industry. Vital experiments on, say, how forests affect water resources and slope stability cannot be turned off and on, nor is there any end-user, other than government, to pay for them.

Yet FRI is better off than the universities, which FRI director Colin O'Loughlin describes as "poorly funded and getting worse". A serious problem, he says, is that "the anticipated expansion of forestry is not being catered for by the universities". So FRI must send people abroad for PhD training for lack of appropriate facilities in New Zealand.

Alun Anderson

Geology and geophysics

DSIR in the research field

GOD (or the gods that control earthquakes) perhaps sometimes works in favour of man. In 1855, just 15 years after Wellington was settled, New Zealand's mightiest earthquake on record tilted a block of land 30 miles wide and 120 miles long, lifting the shoreline at Wellington some five feet. The result was a nice level stretch of dry raised beach all along the coast. And that, local legend has it, allowed a fortune to be made by a settler who had been contracted to build a road along the shore and now found his work largely done.

The road, running just a few feet above the sea, now conveniently links Wellington, where DSIR's Geophysics Division has its headquarters, to Lower Hutt where the Geological Survey is located. Neither institute attempts the hopeless task of predicting earthquakes, which continue at an average rate of a magnitude 7 quake once in ten years and one of magnitude 8 once a

century. The Geophysics Division does, however, offer "words of wisdom and comfort", as one researcher put it, to a public worried about earthquakes and, more important, carries out seismic surveys at construction sites like dams so that, should the thing fall down, the contractor can at least wave the DSIR survey at the court of inquiry.

Accurate seismic risk evaluation, of course, depends upon good records and statistics of earthquake occurrence, and these the division has amassed in abundance. Sophisticated microprocessor-based monitoring systems have also been developed and will help automation of seismic networks. The division also maintains seismic stations in the South Pacific which forms part of the ocean's tsunami warning system. Altogether the division contains 35 scientists, backed up by an equal number of support staff, whose research covers the whole range of the Earth's natural



dynamic activity. Much mapping has been done, including gravity mapping of the whole country, with an eye to the exploitation of natural resources of coal, minerals, oil and geothermal water. Parts of the survey services can be turned to a profit as can the hiring out of seismograph equipment to oil companies. This in turn helps areas such as volcanology that have nothing to offer industry. The pressure to recover more funds from industry sometimes places geophysics in an odd position: if a construction engineer wants to look at seismic records to help design a

safe structure should he be charged — if so, how much?

The Treasury desire that scientists should be looking for customers for their services also looks strange at the Geological Survey. The geology of New Zealand is extraordinarily complicated and the best way to understand it is through fundamental research. Trying to find out only things that someone might want to use would make progress impossible, nor would geological consultants appreciate it much if government geologists really began to compete with them for business!

The survey's 85 scientists perform the primary function of mapping as well as a range of research projects in petrology, palaeontology, Earth deformation and engineering geology. A major study is running on the Cretaceous and Cenozoic sedimentary rocks of the region, principally because they contain all the known coal and petroleum reserves. Most of the

basins of interest are offshore but the lack of an ocean survey vessel is not a problem — all the companies (mainly big foreign oil companies carrying out commercial survey in the region) are required to deposit data, cores and samples, and seismic tapes with the government. These came geology's way because the survey sells its services to the Ministry of Energy who need expert comment on applications for exploration licences.

Much of the expertise acquired in dealing with the petroleum industry is being passed on to smaller countries in the Pacific region. An invitation-only meeting has just been held on "Government and the petroleum industry" to advise representative geologists from the Pacific islands what to do when exploration companies come looking at the sedimentary basins, containing potential oil reserves, that some of the islands possess.

Alun Anderson

New Zealand's changing agriculture

A GLANCE at the histograms below will tell you that New Zealand's agriculture, responsible for some 80 per cent of export earnings, is in a period of transition. The traditional money earner — the export of meat — is scarcely growing at all.

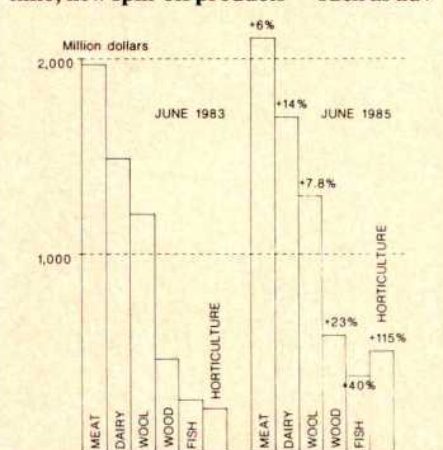
Intelligent diversification in the dairy industry has maintained some growth, and the demand for wool too is "holding up". But real growth is only being seen in fish, helped by the creation of the 200-mile protection zone; wood products, which are just at the beginning of a big leap forward; and horticulture, powered by the amazing success of kiwi fruit.

Just what has happened to New Zealand's traditional exports became clear from conversation with Pat Joyce, looking out from the roof of the Ruakura Agricultural Research Centre, Hamilton, over the acres of experimental land he is responsible for. In quite a few of those acres, deer are grazing in pens. An eager export market has been found for venison and the breed is being improved. Indeed, hunters who used to shoot wild deer from helicopters now bring them back alive for breeding.

The move to deer (along with angora and mohair goats) has come about because of the increasing difficulties of exporting the traditional products of sheep and beef. Most countries have quotas for beef now and, in what could be a big market for sheep, the United States, the Sheep Growers' Association form a powerful lobby. The traditional UK market was, of course, lost after Britain's entry into the European Economic Community.

With these changes comes an ever increasing need for research, firstly to exploit the new opportunities seen in the rapidly growing non-traditional product areas, and secondly to do something about the static areas. Despite the kiwi fruit

miracle, horticulture is not about to replace meat as the main export and new markets have to be found for the traditional products. Already there is much dependence on less-than-stable countries. Half of New Zealand's lamb now goes to Iran. China is taking a large part of the wool exports. But to break into the big US markets, changes have to be made — lamb, for example, would have to be made acceptable for TV dinners. At the same time, new spin-off products — such as adv-



anced milking machines — have to be sold overseas.

These demands come just as the government has announced that the 1985–86 budget for Ministry of Agriculture and Fisheries research is to be cut to \$NZ67 million from an expected \$NZ68 million; the next year it will fall to \$NZ63 million, and then to \$NZ58 million. The shortfall is to be recovered from industry by contract work and charitable services, such as soil analysis. This leaves places like Ruakura with a bit of a problem for they know, even if the Treasury does not, how vital it is to keep the long-term research projects going.

Alun Anderson

Information technology



DSIR in the picture

If all DSIR's scientists were put together they would not match the resources of even one big US or Japanese electronics manufacturer. What opportunities are there then for DSIR's newest division — that for information technology — set up, one hopes inappropriately, on April Fool's Day this year with just 20 scientific staff?

Surprisingly, the answer is "plenty", for the new division is finding a niche appropriate to its size. It has already equipped itself with glossy brochures from a software company that is selling an image analysis package developed by division scientists. And if the enthusiasm of its young director, Peter Ellis, continues to win over the government, New Zealand will soon have a chance to get in on the potentially lucrative satellite image processing market.

The image analysis package, called EPIC, is a flexible system for enhancing digitalized images — it can be used to deblur images, colour different image values, remove distortions, interpolate values and the like. There are a thousand and one uses for such packages, from intensifying details in X-ray images to picking out the number plate of a speeding motorist caught with a video camera.

But what particularly interests the new division is satellite image processing. Much of their expertise was gained in working on Landsat images (bought on large magnetic tapes) and their hope — inspired by some nods from the government — is that a ground station will be built to receive real-time images from SPOT, the French resources satellite to be launched later this year. Later on, other satellites could be tapped — the European Space Agency's ERS-I and then the two Japanese marine observation satellites. Use of SPOT would allow very fine mapping to be carried out as well as real-time crop analysis. Beyond that, if all goes well, there are even ambitions to participate in the design of instruments for use on-board spacecraft.

Most of the division's work is more down to earth. Major effort goes into running the DSIR's computer network and ensuring that the best software is available: here it is careful monitoring of what is available around the world rather than expenditure of original research that counts.

Image processing and the network between them absorb nearly all of the division's resources: what is left goes into projects such as a computerized system for the government printer, and character recognition systems to help with archiving in the National Library.

Alun Anderson

Advisory Council

A watchdog with no teeth

JUST as messengers carrying bad news run the risk of being blamed for the news itself, so central organizations for giving advice on science run the risk of being unpopular when finances are being cut.

New Zealand's National Research Advisory Council (NRAC) is no exception to this rule.

Nobody, it seems, has a good word for the council. The most charitable comment is that "NRAC is not what we need, but it's really not their fault". Indeed, if NRAC's recent record is anything to go by, it has laboured mightily with limited resources to advise a government which has little intention of listening. That may change, for NRAC is likely to be reborn in a new form. Almost nobody doubts that some such body is needed, and badly.

NRAC was set up in 1963 to give advice on the planning of scientific research, including the setting of priorities for government departments. Three kinds of obstacles have, according to government critics, made it impossible for NRAC to fulfil this role. First, the organization is very small (12 committee members) and relies on busy people, with commitments elsewhere, who cannot deal with the multitude of tasks that come their way. Second, it has never had a professional technical secretariat, but has to beg, borrow and steal personnel from the Department of Scientific and Industrial Research (DSIR) and other bodies. And finally, the government has tended to ignore it.

The line of communication to government is certainly long: NRAC reports to the Minister of Science (a low-ranking portfolio), who then reports to the cabinet, rather than directly to the Prime Minister. And again, the minister's portfolio includes responsibility for DSIR, so there is a confusion of his role as an overseer of the whole of science and as a director of one major science body.

To point out that there are shortcomings in the system is to say nothing new. NRAC's role has been under some kind of review for four years; self-criticism has been rife for longer. In July last year, the Probine report on the means by which the government obtains advice on science and technology policies recommended that NRAC should be revamped as a Science and Technology Council which, like those of other countries, would report directly to the Prime Minister. That report also asked for a new research council, with funds independent of those of the UGC, which could harness "under-utilized talent and equipment in the universities".

No action has yet been taken; NRAC is left "hanging in the air" as one member puts it, although action of some sort is still expected.

Uncertainty and criticism there may be, but NRAC has not been inactive. Its 1984

"Science and Technology Plan" surveyed the whole of New Zealand's research effort, and has not escaped criticism. Those involved in applied research say that it states the obvious, while those in the universities are offended that "The role of the universities" occupies a mere two of 166 pages. But the report was intended to be "more of a review than a plan"; a second plan is to appear shortly and seems considerably more vigorous.

To compile the 1985 plan, the "users" of science (manufacturers and primary producers) were approached to discover their needs; merely making this effort seems to have awakened considerable interest among the producers. Scientists also had an opportunity to air their views at a large May conference on "Science and Technology for Development", which generated several inches of documents from indus-

trialists, technologists and academics on the remedies for New Zealand's woes. No panaceas or plans for radical reform emerged, but plenty of heated argument.

The head of NRAC, Alan Mackney, is not averse to expressing a few strong opinions either. On the government's attempts to recover a fixed percentage of funds by the user-pays principle, he says that "an arbitrary amount to be got without rhyme or reason" just does not work", and that if the user, who often does not know what he needs, is to decide what must be done, New Zealand will be "frozen into pre-technology".

The annual reports of NRAC, too, contain endless recommendations that science should be taken more seriously. But until NRAC is reborn as a body that represents all scientists and technologists (and the universities have been conspicuous by their absence) and until it has real teeth (access to decision-makers and money), its activities will continue to be all bark and no bite.

Alun Anderson



Geothermal energy

Looking for a crock of gold

NEW Zealand can proudly point to one area of research where it is a world leader: the chemistry and physics of geothermal systems. Confounding the politicians who are now demanding a short-term pay off from science, the long-term accumulation of expertise in geothermal research has allowed two New Zealand consulting firms to win big foreign contracts for the design of geothermal power plants. In 1958, New Zealand became the first country in the world to build a geothermal power plant operating on the mixture of steam and hot water that is found in the vast majority of the world's geothermal fields. Part of the steam was originally intended for a UK heavy-water project never carried through. Fortunately, the flexibility of the design for turbine operation has meant that the power plant, at Wairakei, is still operating with utter reliability producing 5 per cent (160 MW) of the country's electricity.

To generate the electricity, one tonne of fluid a second is taken from boreholes drilled one to two kilometres into the ground. The area has become a mecca of geothermal understanding. There have been some surprises. As steam has been taken out of the ground, the water table has fallen as much as 15km away, and the surface has subsided, in one place by 0.4m a year. A nearby tourist attraction that used to be called Geyser Valley is now known as Geothermal Valley because the geysers have turned into mud pools. On top of that, an unhealthy level of arsenic has been found in the geothermal water discharged into the river that flows down to the town of Hamilton.

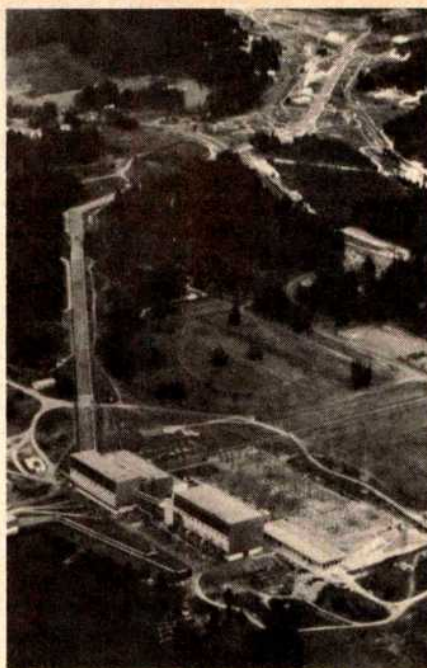
None of these problems has proved as serious as might appear, however, while the expertise gained and the basic research done on the geothermal system have paid dividends. Now two geothermal companies, GENZL and KRTA, contain-



An active geothermal field in central New Zealand.

ing many researchers formerly with the Department of Scientific and Industrial Research and the Ministry of Works, have taken a large share of contracts in the Philippines, which is now second only to the United States in the number of its geothermal power plants.

The latest surprise at the Geothermal Research Institute has been for the geochemists: the insides of some of the borehole pipes have been found to be coated with gold. What seems to be happening is that, in the geothermal system, gold forms a complex with dissolved hydrogen sulphide which precipitates when the pressure drops. The implications are important. Hitherto the amount of gold deep in the system has been inferred from that left in the geothermal water, but with the amount now known to be deposited added to that, it seems that, deep down, the hydrothermal water is close to saturated with gold. This strengthens the case for thinking that the geothermal system beneath Wairakei is a living model of fossil geothermal systems that are now being actively mined for gold and study could thus aid prospectors. The work on gold deposition is not yet published, but Richard Henley's book *Fluid-Mineral Equilibria in Hydrothermal Systems* is already a modest bestseller, going into a second printing after nine months.



Capable of supplying 5 per cent of New Zealand's electricity, the Wairakei geothermal power plant.

While prospecting for gold may be in fashion, prospecting for steam is in the doldrums in New Zealand. The black art of prospecting has been refined by the Wairakei centre so that the chances of a

borehole turning out to be successful are very high indeed. The technique is a combined operation between geologists, who do the basic mapping, geophysicists, who use electrical resistivity and seismic techniques to map the reservoir, and geochemists who reckon to predict subsurface temperatures from analysis of fumarole steam. (Asking which group contributes most is a sure way to begin a brawl.) But there is little chance that new power plants will be built in New Zealand. Instead, the tricks of the trade are being transferred to others at a special Geothermal Institute, supported by the United Nations, at Auckland University.

Just why New Zealand has been able to keep the lead in this area was neatly explained by Jeff Hedenquist, a geochemist at Wairakei, and himself from the United States. In New Zealand, all geothermal work is done by government agencies and information is freely available; in the United States, by contrast, there is free competition for contracts which sometimes means that one system is drilled by several companies without any sharing of information. The result is that it may be difficult to understand the system as a whole. Perhaps the New Zealand government should bear this in mind before pushing ahead with its plans for "liberalization".

Alun Anderson

Chinese gooseberries and tree tomatoes lead the way

"It's a miracle", said one Australian agriculturalist, "that the New Zealanders have been able to sell that horrible looking, tasteless fruit". He was uncharitably referring to the kiwi fruit, a few years ago totally unknown and now on supermarket shelves from Tokyo to London, this year worth \$NZ220 million in export sales.

The curious thing about the kiwi fruit is that it really has nothing at all to do with New Zealand. Its first mention is in a poem written in the Tang dynasty: the kiwi fruit is Chinese, and came to New Zealand only by chance. At the turn of the century, in Yiohong, on the Yangtze River, a small colony of missionaries and traders learned to cultivate the fruit as a local novelty. Among the missionaries were three young Church of Scotland ladies from New Zealand. One of them had an adventurous sister who came out to China for a holiday, and took back with her seeds of the "Chinese gooseberry" which she passed on to a local sheep farmer with an interest in novel plants. All the kiwi plants in New Zealand can be traced back to these first specimens.

As the Chinese gooseberry, the plant was never a commercial success. Turners and Growers, New Zealand's best known fruit merchants, tried to sell it in California without success. At a meeting called to remedy matters the chairman of the board, in a moment of inspiration, rejected a suggestion that the fruit be renamed the

"melonette" and pronounced, "this is the land of the kiwi and it shall be the kiwi fruit". The rest is history. Exports have grown from \$NZ4 million in 1976 to \$NZ220 million this year. From the number of kiwi fruit plants now going into the ground, it is predicted that by 1990, the industry will be worth \$NZ740 million. If that happens, horticulture will account for 13 per cent of New Zealand's exports com-

It's from the U.S. Navy—can we sell the idea of nuclear warships?



pared with 5 per cent now and just 2 per cent five years ago.

So Rod Bielecki, director of the DSIR Division of Horticulture and Processing, wonders if the government knows exactly what it is doing. The Treasury is squeezing research funds and demanding that more money be recovered from industry, just as

an important new export sector is being born. Behind the kiwi come a host of other exotic fruits being readied for the export market: fijoas, tamarillos (no longer called "tree tomatoes"), persimmons, Asian pears and pepinos, already not uncommon in Tokyo supermarkets.

To make them into real winners, there will have to be selection programmes to improve taste, cultivars specially suited for New Zealand conditions, research on plant pests and on the improvement of keeping qualities. Bielecki feels the government is far too keen to transfer the burden of research to industry when, if anything, increased government research in this sector would pay dividends.

To those who say that the money should be recovered from the farmer, Bielecki replies that most horticultural end-users are small single farmers, who need one central research institute. Nor does Bielecki believe the government understands the role science plays in generating wealth. It was one of the division's predecessors which identified kiwi fruit varieties that keep long enough for them to be exportable. More recently, the division was called in when an unexpected frost hit before harvesting was complete, and it seemed that much of the crop might have to be dumped. As fruit growers expand into less favourable land, and as the range of fruits increases, there will be many other problems to solve.

Alun Anderson

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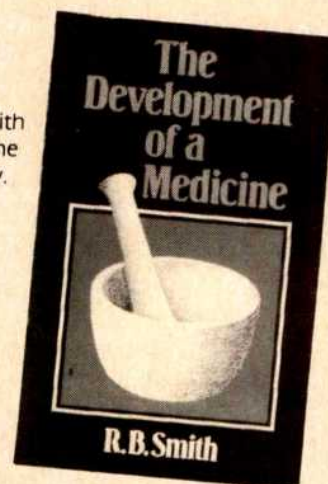
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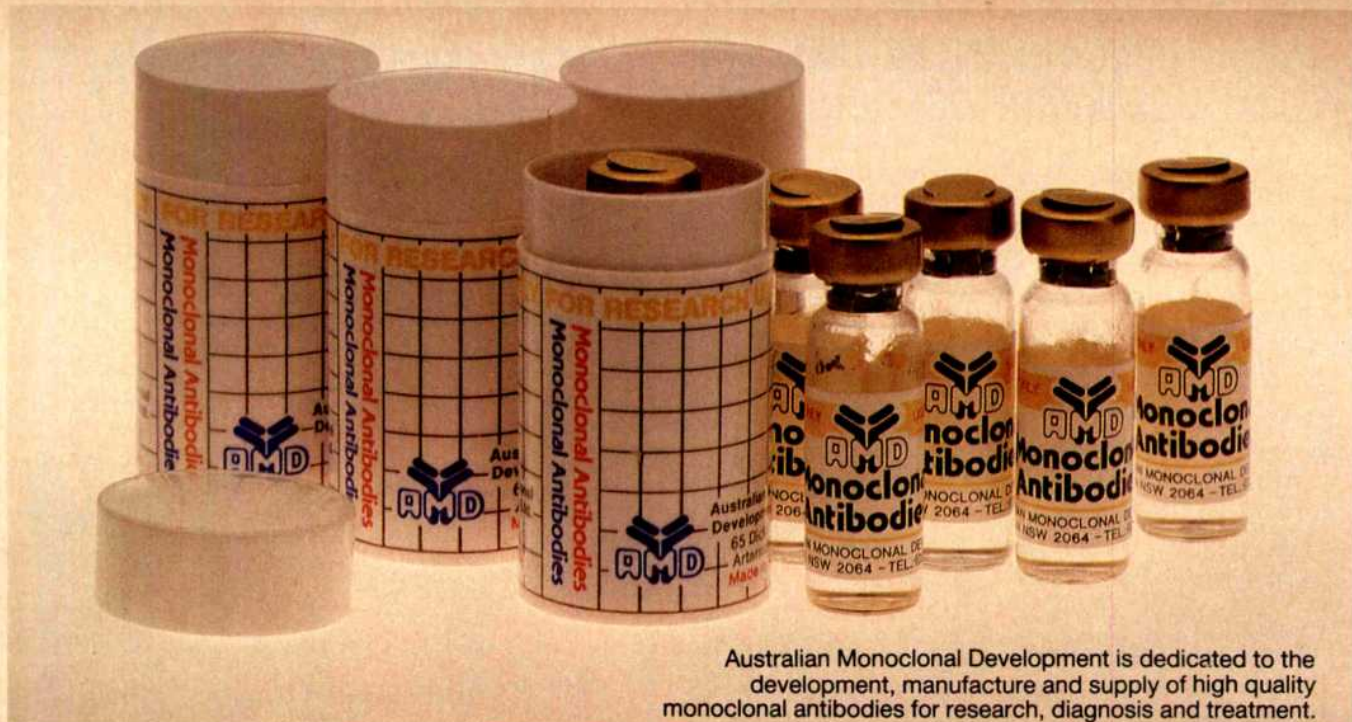
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Reader Service No.3

Stefan Marinov wins friends

The suggestion that there are systematic departures from the strict requirements of special relativity has been persistently put forward by Dr Stefan Marinov. There is a case for repeating his experiment.

STEFAN Marinov is a remarkable iconoclast who is convinced that Einstein's special theory of relativity is mistaken. Bulgarian by origin, Marinov has been, for all practical purposes, exiled from his homeland, but it is by no means clear whether his opinions on relativity or on the proper conduct of the Soviet leadership are chiefly responsible.

From his new home in neutral Austria, Marinov has been at loggerheads with several scientific journals, *Nature* among them, because of their refusal to publish any but a small part of his work, which now also includes a purported demonstration of a *perpetuum mobile*.

Marinov's frustrations with the journals have recently seemed to get the better of his judgement. Last year, angered by *Nature's* refusal to publish a group of manuscripts, Marinov was for a time threatening to immolate himself in the square outside the British consulate at Genoa. Later, he threatened to do the same outside the British embassy in Vienna, but fortunately settled for a press conference instead. Those who have had dealings with Dr Marinov can have been left in little doubt that he is passionately convinced of the correctness of his position on special relativity. Quite apart from his apparent willingness to take his own life, it is clear that his waking hours are almost all spent in correspondence, much of it unfortunately fruitless, about this theory and about his purported demonstration, by means of what he calls the "coupled-shutters experiment", that the velocity of light is not, as Einstein's theory supposes that it would be, equal in all directions (isotropic) in all frames of reference.

Now, it seems, Marinov has won some influential friends. The issue of *Physical Review Letters* for 8 July (55, 143; 1985) contains an article by A.K. Maciel and J. Tiomno from the Brazilian National Science and Technology Research Council at Rio de Janeiro which at least puts Marinov's objections to special relativity in a context in which they can be grappled with. Moreover, what Maciel and Tiomno say is certain to have an important bearing on the classification of all possible experimental tests of special relativity.

Plainly, when there is such a mountain of experimental evidence that the general features of special relativity are borne out in practice, it would be foolish in the extreme to assert that special relativity is just plain wrong, thereby implying that the

time has come to return to newtonian mechanics; after all, objects do not travel faster than light in any circumstances observable, while time dilation (as defined by Lorentz) is a fact of life and can be measured, for example, by the difference between the average lifetime of an unstable particle in motion and at rest. So if there are violations of special relativity, they are at most weak violations, departures from what is not strict orthodoxy and not flat contradictions of it.

But what weak violations of special relativity are neither trivial nor absurd? Borrowing from some work due to H.B. Ives in the 1930s, Maciel and Tiomno argue that it is prudent to disallow violations for electromagnetism, for independent particles in uniform motion and the other more elementary phenomena used as illustrations of special relativity, now even in the elementary textbooks. But what about the possibility that the behaviour of rigid bodies is not accurately accounted for by the Einstein-Lorentz transformations of coordinates, and that a rigid body remains rigid — the separation of fixed points remains constant — however it is moving?

The formal realization of this state of affairs seems not particularly elusive, although it is not possible to modify special relativity in any meaningful way without reintroducing the newtonian concept of absolute time and space, or the idea that there may be some preferred frame of reference such as that of the microwave background. Explicitly, if x, y and z are cartesian coordinates in that absolute frame of reference, and t is the time, and if primed quantities are the same coordinates in a system moving at velocity v along the x -axis, the transformed coordinates are exactly those of special relativity except for t' , which is given (according to the formalism of Maciel and Tiomno) by $t = \gamma t'$, where $\gamma = (1 - v^2/c^2)^{-1/2}$ and not the conventional Lorentz transformation in which xv/c^2 is added to t' .

The calculations are not in themselves particularly difficult. It emerges simply enough that rigid rotating bodies remain rigid in the new system, and in the particular sense that the apparent angular velocity of all fixed points is a constant (if the angular velocity of the whole object is constant). The velocity of light is isotropic in the absolute system against whose coordinates the rotation of the rigid object is defined, while both time dilation and

the Lorentz-Fitzgerald contraction (of lengths in the direction of relative motion) persist. What is sacrificed is the isotropy of the velocity of light in the rotating system.

The interest of what Maciel and Tiomno have done is that they can sort the several published tests of special relativity by the likelihood that they will yield a stringent test of the weakly violated form of special relativity defined by their transformation. The classical test of special relativity first carried out by Michelson and Morley and since repeated several times does not meet the test. The version of the experiment due to Joos (published in 1930) in which a Michelson interferometer was rotated on its turntable once every ten minutes or so, classically one of the better verifications of the isotropy of the velocity of light (among two orthogonal directions), will not suffice, although a more rapidly rotating turntable might meet the need. The simulation of the Michelson-Morley experiment in which two hydrogen masers are used as the source of radiation moving in orthogonal directions offers more hope of providing a stringent test of the modified transformation equations. And so, Maciel and Tiomno say, does Marinov's rotating-shutters experiment.

The principle of that is simple enough, a kind of Fizeau measurement of the velocity of light except that the objective is to make a direct comparison of the velocity of light in two opposite directions. Two disks with identical perforations are mounted parallel to each other at opposite ends of an axle and light from a laser is directed (by means of a beam-splitter) through the perforations of the disks in each direction. Marinov claims that his results, most recently obtained with home-made equipment at Graz, demonstrate that the velocity of light is not the same in all directions. He even claims to have been able to detect the velocity and direction of the Earth's movement through absolute space and time.

None of this proves that there is anything wrong with special relativity. It is merely a pointer to the kinds of tests that would be necessary to demonstrate a particular (and "weak") violation thereof. Maciel and Tiomno merely say of the Marinov experiment (as of the others on their list) that it "should be repeated even if to prove it wrong". It will be interesting to see how many correspondents write in to claim that that has been done already.

John Maddox

Haemoglobin structure

A clam with a difference

from Max Perutz

ALL vertebrate haemoglobins are tetramers made up of two pairs of unlike polypeptide chains, termed alpha and beta, each chain forming a basket for one haem. But on page 227 of this issue W. E. Royer Jr, W. E. Love and F. F. Fenderson demonstrate that haemoglobin of the clam *Scapharca inaequalis* has a quite different form of subunit assembly.

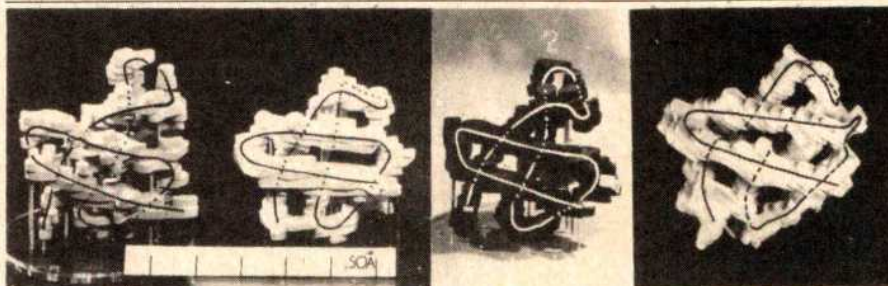
In vertebrate haemoglobins, the alpha chain is coiled into seven helical and six non-helical segments, and the beta-chain is coiled into eight helical and six non-helical segments. The tertiary structures of both the alpha and beta chains are similar to each other and to myoglobin, and the residues lining the haem pockets are largely homologous. The vertebrate tetramer has pseudo-tetrahedral symmetry with one true dyad axis running through a central water-filled cavity and two pseudo dyads at right angles to each other and to the true dyad. Assembly occurs in two stages: first, one alpha and one beta chains form a tightly bound dimer; and second, two such dimers combine to form a tetramer. The allosteric change between deoxyhaemoglobin and oxyhaemoglobin consists in a large rotation and slight translation of the two alpha-beta dimers relative to each other.

The haemoglobins of two primitive vertebrates, lampreys and lungfish, are monomeric when oxygenated and oligomeric when deoxygenated; the monomer has a tertiary structure similar to myoglobin. The same is true for all the invertebrate haemoglobins whose structure has been determined and for the haemoglobin found in the root nodules of leguminous plants, suggesting that the myoglobin fold is a universal pattern (see figure) that has evolved to provide the surround for an oxygen-carrying haem.

A suspicion that the quaternary structure of invertebrate haemoglobins may be different from that of vertebrates arose from the observation that one of the larval haemoglobins (component X) of the fly *Chironomus thummi thummi* has a marked Bohr effect coupled to a dimeric structure at pH 5.5 and to a monomeric structure at pH 7.0. Bohr effects probably arise from the ionization of histidines, but no histidine in this haemoglobin is at a position corresponding to subunit contacts in vertebrate haemoglobins.

Royer *et al.* now show that haemoglobin of the blood clam *S. inaequalis* has a tertiary structure similar to myoglobin, but with one additional helix at the amino end which lies parallel to the carboxy-terminal helix H and brings the terminal amino group close to the terminal carboxy group. It is in its quaternary structure that

the clam haemoglobin is exceptional. Two like monomers assemble to form dimers, not by contact between helices G and H as in vertebrates, but by contact between the



The universal tertiary structure of, from left to right, sperm-whale myoglobin, horse haemoglobin alpha chains, horse haemoglobin beta chain and haemoglobin of the clam *Glycyca dibranchiata*. In each picture the amino end is on the left.

haem-linked helices E and F, so that the haem pockets lie on the inside of the tetramer, as Pauling once imagined, rather than on the outside, as in vertebrates. Two chemically different dimers join to form a tetramer; as far as one can make out from Fig. 2 of Royer *et al.*, the tetramer is formed by contact

between helices A, and between the non-helical segments NA and GH. The dyad axes of each of the individual dimers make angles of 75° with the dyad joining the two dimers, so that there is no tetrahedral symmetry.

The structure solved is that of the carbonmonoxy-derivative which is probably isomorphous with the oxy-derivative. Oxygen binding is cooperative with a Hills coefficient of 2.1, and loss of

oxygen is linked with polymerization of the tetramer. It will be interesting to see if solution of the deoxy-structure reveals the mechanism that triggers this polymerization. □

Max Perutz is at the Laboratory of Molecular Biology, MRC Centre, University Medical School, Hills Road, Cambridge CB2 2QH, UK.

X-ray astronomy

A new breed of oscillator

from N. E. White

THE first short timescale periodic oscillations from a bright galactic centre X-ray source (GX5-1) are reported by van der Klis *et al.* on page 225 of this issue. These quasi-periodic oscillations (QPOs) probably indicate the presence of an accreting neutron star that is rotating with a period of a few milliseconds. This discovery may provide timely support for recent models for the evolution of low-mass X-ray binary stars (LMXBs) into millisecond radio pulsars. Furthermore, the oscillations themselves display intriguing characteristics requiring some ingenuity from theorists seeking to explain them.

An accreting neutron star will only be seen as an X-ray pulsar if its surface magnetic-field strength is high enough to tunnel the inflowing material from its companion star onto the magnetic pole. The spin-down properties of radio pulsars suggest that neutron star magnetic fields decay on a timescale of 10^6 – 10^7 years. If neutron stars are born with the same surface field of 10^{12} – 10^{13} Gauss, then those located in the X-ray binaries should show a range of field strengths depending on their age. X-ray pulsars are usually associated with young population I objects (OB stars). In contrast, the older population II

LMXRB systems (where the mass-losing star is of a late spectral type) rarely contain X-ray pulsars.

Any material that flows between components in a binary system must lose angular momentum before it can fall onto a compact object. It may form an accretion disk which will mediate the flow and, by exerting torque, will spin the neutron star up to equilibrium period at which its rotation period is equal to that of the innermost keplerian period of the disk. If a strong magnetic field is present this is determined by the radius at which the magnetic pressure disrupts the disk; for a typical X-ray pulsar with a field of $\sim 10^{12}$ Gauss the equilibrium period is a few seconds. If the field is less than 10^8 Gauss this disk will extend to the neutron star surface and the equilibrium period is ~ 1 ms. It takes only 10^6 – 10^7 years to spin a neutron star up to this period.

This scenario has encouraged the search for millisecond periodicities from LMXRBs but with no success until van der Klis *et al.*, using the European Space Agency X-ray observatory (EXOSAT) because it is probably more sensitive to millisecond periodicities than previous X-ray satellites, searched again for milli-

second pulsations from the brightest of the LMXRB — the galactic bulge sources.

The oscillations found from GX5-1 are not at a single frequency but rather show a distribution of frequencies whose centroid and width are correlated with luminosity. An increase in luminosity by a factor of only 1.4 causes the average frequency to increase from 20 to 40 Hertz and the frequency spread to increase from 4 to 12 Hertz.

The large variation in frequency eliminates the possibility that QPOs directly reflect the spin period of a neutron star. Any model must explain the very strong dependence of oscillation frequency with luminosity. Of the various possibilities discussed to date, the most promising seems to be an instability at the edge of a magnetosphere. Magnetospheric models use the fact that an increase in mass-transfer rate (luminosity) will cause a compression of the magnetosphere such that the characteristic keplerian period at the inner edge of the accretion disk becomes shorter. This gives a frequency/luminosity dependence that is in the correct sense and, with some refinement described elsewhere in this issue², the correct magnitude. These models predict a neutron star rotation period of roughly 10 milliseconds and a surface magnetic field in the order of 10^9 Gauss for GX5-1.

The orbital periods of high-luminosity LMXRBs ($\sim 10^{38}$ erg s⁻¹) such as GX5-1 are in general unknown, but in the two sources where they have been measured, ScoX-1 and CygX-2, are found to be 0.7 and 9 days. In these cases, only an evolved giant companion star can fill its Roche lobe, such that the mass transfer will be driven by the evolutionary expansion of the giant (causing the stars gradually to spiral apart). In such a system the mass transfer ceases when the mass-losing star becomes degenerate.

It has been pointed out that the compact object may begin as a white dwarf, but because of the extra mass accreted, is pushed over the Chandrasekhar limit to collapse into a neutron star³. This could lead to a young X-ray pulsar in an LMXRB. The magnetic field then decays on a timescale that is a factor of ten shorter than the evolutionary timescale. It is plausible that if the neutron star is formed close to the end of the mass-transfer phase then a sizable magnetic field will be left and a millisecond radio pulsar born.

The discovery last year of a 6-millisecond pulsar that has a degenerate companion in a 120-day orbit caused much excitement because such a binary is expected as the end-point of this scenario⁴. The parameters deduced from the magnetosphere model for the underlying neutron star in GX5-1 are similar to those determined for the 6-millisecond radio pulsar. This may be a coincidence — if the 6-millisecond pulsar evolved from a LMXRB it would presumably have looked very like GX5-1 does today.

The magnetosphere model, however, has at least two worrying aspects. First, it is perturbing that the underlying rotation period is not detected. Although there may be ways around this problem (for example, smearing of the pulses in surrounding material) it remains unsolved. Second, the nature of the magnetosphere model allows it to reproduce any frequency/luminosity relation if the dynamic range of the luminosity variation is small. This makes the model extremely flexible, but means that it is not uniquely specified by the data. Other possible models for QPOs are not sufficiently developed to make specific predictions. One suggestion (based on a similar phenomenon that has long been known from the cataclysmic variables) is that the neutron star does not have a substantial magnetosphere, and that the oscillations are generated in the large velocity gradient of

the boundary layer between the disk and the neutron star surface⁵.

Following the GX5-1 result, QPOs have been found in two other low mass X-ray binary stars of high luminosity and long orbital period, ScoX-1 (ref.6) and CygX-2 (ref.7). QPOs had previously been missed in these two sources because observers were searching for single peaks in their power spectra rather than broad humps. □

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Archaeology

Industrial revolution in York

from Richard Hodges

RESCUE archaeology has produced a wealth of information about town and country settlements in Britain from Neolithic to modern times¹. These archives constitute a remarkable documentation of our heritage. However, in a phase of stock-taking imposed by current financial constraints it seems appropriate to ask how we have benefited from these excavations.

Most rescue excavations have been concerned with historical archaeology. Redevelopment of inner city centres has allowed deep-stratified excavations of medieval, Anglo-Saxon and Roman layers. The same has happened in the countryside where deep ploughing has obliterated very many remains of Roman-period, Anglo-Saxon and medieval villages and farms. One consequence of this destruction is that there now exists an accurate measure of all these types of historical settlements. The prospects of writing a history based on material remains

has not been fully appreciated until now. To illustrate this point I discuss here some recent excavations which reveal what the written sources do not — the first English industrial revolution.

Rescue excavations reveal the scale of change that occurred in about AD 900. The



Excavations at the Coppergate dig, York. (Courtesy of Jorvik Viking Centre, Coppergate, York YO1 1NT, UK).

Viking dig at Coppergate, York (see figure) provides probably the most perfect illustration of the industrial explosion which occurred at the beginning of the tenth century². Rescue excavations beneath the modern tenements at Coppergate have uncovered a collection of workshops laid out at right angles to the modern street grid. The timbers surviving in these structures are dated by dendrochronology to about AD 900. Much rubbish, associated with the occupation of the associated tenements as well as their workshops, provides some measure of the revolution in craft production in Viking York. As Richard Hall shows³, these tenements were planned on largely unoccupied ground; coopers, moneyers and smiths worked here in the tenth century. Botanical evidence as well as the microscopic remains of beetles and other insects betray the living conditions of the new urban class, whereas excavations elsewhere in the city show a high mortality rate among children and a shorter life-span for women by comparison with rural standards of the age³.

Coppergate, like many smaller rescue excavations in York, confirms the decline of the Roman town in the fourth century, as well as the general desertion of the centre between about AD 400 and 900. The urban take-off, focused on these earlier administrative settlements, occurred in around AD 900, and with it York was transformed. Rescue excavations in Viking towns such as Colchester⁴, Lincoln⁵, Northampton⁶ and Thetford⁷ have documented the transformation from a rural elite centre to a vibrant market town during this period. At Northampton, for example, the middle Saxon minster and the Mercian palace have been located and excavated; both were subsumed into the matrix of a fast-expanding tenth century market⁸. Indeed, the mark of the Viking age in eastern England are these markets and the development of craft production.

New markets are also a prominent feature of most southern English towns at this time. Their foundation may be considered the real achievement of King Alfred the Great. Recent excavations in Canterbury, Chichester, Exeter and London⁹, as well as Martin Biddle's famous excavations in Winchester¹⁰, all conclusively demonstrate that these places had lain more or less vacant since Roman times until they explode into life once more at the end of the ninth century. Most medieval street systems stem from this time. Thousands of tons of gravel were quarried and laid in an operation that was as complex as any by Roman land surveyors. Decaying fortifications were refurbished, new tenements were designed and, as in York, craftsmen were starting in business almost overnight, producing a great variety of commodities for the surrounding rural markets. Alfred's kingdom was on its way from being an underdeveloped economy to one which, seventy years later, was one

of the richest in western Europe.

The industrial revolution was necessarily supported by changes of comparable scale in the countryside. Rescue excavations at Raunds in Northamptonshire¹¹ have charted the emergence of the medieval village, and it is a pattern now noted in many other places. The middle Saxon farm was evidently transformed between about AD 850 and 950, when it became part of a collection of farms focused for the first time on a manor and a parish church. Indeed, the manor and parish church seem to be features of the revolution as local lords and the Church tried directly to control agrarian resources. In the tenth century the number of rural communities expanded rapidly.

The scale of this revolution is mainly a measure of late Saxon and Anglo-Scandinavian government. Powerful centralized kingship was a feature of England from the Viking age to the Norman conquest and in complete contrast to the diffused authority of most continental kings¹². The authority and the capacity to introduce markets and the market principle to Anglo-Saxon England is a measure of political control¹³; to execute it on the scale witnessed in these excavations is a measure of the nation's wealth at that time.

A generation of rescue archaeology, therefore, has shed light on a momentous movement. With the agrarian and industrial revolutions of Alfred's age the form of present-day landscapes and townscapes in

Britain took shape. But archaeology on this scale offers more than a chance to reassess local history; it makes it feasible to calibrate the management of resources and to examine the social implications of such strategies. It is certain that the identification of this first English industrial revolution is just one example of the opportunities to rewrite the history of the Roman and later medieval periods. Rescue archaeology has convincingly shown that mainstream history can be studied in the field as well as in the archive. □

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Malaria vaccine

Components of a cocktail

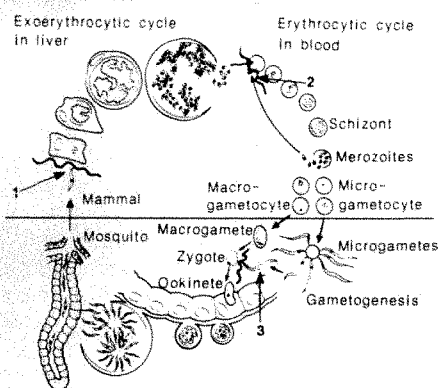
from F.E.G. Cox

IN THE wake of spreading drug resistance, the search for a realistic malaria vaccine has taken on a new urgency — most workers now agree that this will have to consist of a 'cocktail' of antigens or, as the World Health Organization puts it, a multivalent vaccine. Rapid progress has been made in identifying and characterizing potentially protective antigens from malaria parasites at various stages of their life cycle. A major antigen of the male gamete of the rodent parasite, *Plasmodium yoelii*, which can elicit antibodies that block transmission of the gamete from mice back to the mosquito vector, is described on page 258 of this issue¹. The human equivalent of this antigen may eventually be an important constituent of a vaccine but needs to be considered in the context of all the other antigens contending for this final selection.

The complex life cycle of the malaria parasite (see figure) begins when sporozoites are injected directly into the bloodstream by a mosquito. The sporozoite is, therefore, an obvious target for immunization and the major sporozoite antigen of the human malaria parasite,

Plasmodium falciparum, has already been expressed in *Escherichia coli*² and has proven immunogenicity³. Equivalent antigens have been used to immunize laboratory animals with considerable success. Unfortunately sporozoites circulate for only a few minutes before entering liver cells where they undergo a massive phase of multiplication and acquire new antigenic specificity. The products of multiplication, merozoites, which are again antigenically distinct, invade red blood cells to initiate the most destructive multiplicative phase of the life cycle.

Relatively little is known about the stages preceding this initial red-cell invasion but the intraerythrocytic stages have been intensively studied. Many antigens have been detected and the potentially important ones appear on the surface of the dividing parasite (schizont) at a late stage and on the merozoites resulting from division. These antigens, which vary from species to species and even within species, have relative molecular masses (M_s) between 190,000 (19K) and 23K (ref.4). Purified and partially purified antigens have been used with some suc-



The life cycle of *Plasmodium* spp. in mammals. The main targets for the immunological control of malaria are: (1) anti-sporozoite (prevents entry into liver); (2) anti-merozoite (prevents entry into red blood cell); (3) anti-gamete (prevents fertilization). Wavy line indicates blocking. Redrawn from Cox, F.E.G. *Modern Parasitology* (Blackwell, Oxford, 1982).

cess to immunize laboratory animals; the protection seems to involve blocking attachment and invasion by the merozoites of fresh red cells. The merozoites themselves possess several distinct antigens associated with either the surface or internal organelles. The gene for at least one of these surface molecules has been cloned and expressed in *E. coli*⁵ and a surface antigen has been used to immunize monkeys with partial success⁶. Some of these antigens may be involved in attachment of the merozoites to the red cells.

The final stage of the life cycle begins when merozoites enter red blood cells and develop into sexual stages or gametocytes. These remain in the red cells while in the blood and possess yet different antigens. Normally there is no immune response against the gametocytes, but once they re-enter a mosquito, they leave their protective red cells as gametes and become vulnerable to any antibody that is also taken up with the blood meal. These are the stages studied by P. G. Harte, N. Rogers and G. A. T. Targett¹. Using a rodent model, *P. yoelii*, they have identified on the male gamete a major antigen with *M*_{42K} and several minor antigens of 67, 59, 57 and 35K. Mice were vaccinated with these purified antigens and later infected with *P. yoelii*. The infections progressed as normal and gametocytes formed. However, when mosquitoes were fed on the mice they failed to become infected. The mechanism seems to involve agglutination of the male gametes and the prevention of fertilization. The effects of immunization were dramatic; the parasite load in the mosquitoes was reduced by 99 per cent following two immunizations.

There is every reason to expect that this kind of vaccination will be successful in primates, particularly as earlier work with crude antigens has already been confirmed in monkeys. These antigens, however, are not the only ones associated with the gametocytes and gametes; antibodies against the other antigens block

various stages of development and one even reduces the number of gametocytes in the blood⁴.

The real problem with human gametocyte or gamete vaccines is that they are specific to these stages and do not affect the course of the infection. This means that, for ethical reasons, they could not be used on their own — hence the need for a cocktail of antigens that would at least ameliorate the progress of the disease in man. Another problem is that there is still no rigorous mathematical analysis of the efficacy of such vaccines in the reduction of malaria transmission, so we really have little idea about how effective this method of preventing transmission might be.

There are problems other than the ethical ones. Although the emphasis has

been on antigens and antibodies it is clear that antibody-independent mechanisms are also involved in immunity to malaria⁷. At one time it was thought that a sporozoite, a merozoite and a gametocyte antigen mixture might do the trick but it now clear that the cocktail must be very complex indeed. □

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Mathematics

The Bierberbach gambit

from Ian Stewart

THE theory of complex functions was the centrepiece of nineteenth century mathematics and is still of fundamental importance. It has been worked over thoroughly by generations of mathematicians, and one might imagine that everything worth knowing is now known; but in fact many outstanding problems remain. Among them was a conjecture in geometric function theory made by Ludwig Bierberbach in 1916, which bounds the coefficients in the Taylor series of a complex analytical function satisfying certain conditions.

Despite a sustained attack, the conjecture was verified only in a few special cases until last year when Louis de Branges obtained a complete proof, now available in preprint form (de Branges, L. *A Proof of the Bierberbach Conjecture* Mathematics Department, Purdue University). His method is a development of one used with partial success by Karl Löwner in 1923, and the final gap is filled by an inequality proved by Richard Askey and George Gasper in 1976, in the apparently unrelated (and unfashionable) field of special functions. Initially, de Branges's claim was greeted with scepticism. The tale casts an interesting sidelight on the sociology of mathematical research, as well as its content.

Geometric function theory, as its name suggests, deals with the geometry of transformations of the complex plane. It has its origins in the discovery that the transformations obtained from complex analytical functions are conformal, that is, do not change the angles between curves. Let the unit disk be the set of all complex numbers $z = x + iy$ with $x^2 + y^2 < 1$, and let $f(z)$ be an analytical function. Then f can be thought of as a way of deforming the disk, moving each z to its image $f(z)$. What shape can this image be? According to the

Riemann mapping theorem, anything! (More accurately, anything that is topologically a disk; that is, a simply connected open set.)

The image of the disk may overlap itself (and the Riemann mapping theorem must be stated with due care in that case), but there is an important class of functions f for which it does not. Such an f is said to be univalent on the unit disk. At this point the hint of a connection between topological and metric properties of f arises. The larger the image of the disk under f , the larger, in some sense, f is. The largest univalent function should transform the disk into the largest possible image, namely the entire complex plane. (Actually, for technical reasons, it is necessary to slit the plane along a line segment.) Does this statement make any kind of sense, and if so, what is this largest univalent function? (It looks as if it ought to be interesting in the sense that extreme cases always are.) An attractive guess is the Koebe function, $z/(1-z)^2$, which has the required properties. This has the power series expansion $z + 2z^2 + 3z^3 + \dots + nz^n + \dots$ and it occurred to Bierberbach that its extremality might be reflected in its power series. If so, the n th coefficient in the power series of a univalent function should be no larger than n . In other words, metric extremality should follow from geometrical extremality. It is an appealing, natural and simple idea; the sort of thing that ought to be possible to decide one way or the other without much trouble — but, in fact, a proof turned out to be decidedly elusive.

More precisely, consider a complex power series of the (normalized) form $f(z) = z + a_2z^2 + a_3z^3 + \dots$ which is univalent in the unit disk. The Bierberbach conjecture is that $|a_n| \leq n$ with equality holding only for a rotation of the Koebe function $f(z) = z/(1-\omega z)^2$ where ω is a constant of

absolute value 1. (The circular symmetry of the disk forces us to admit rotations as well as the Koebe function itself.) Until last year the conjecture was known to be true only for $n \leq 6$; this was proved by Bierberbach himself for a_2 , by Löwner for a_3 , by P. R. Garabedian and M. Schiffer in 1955 for a_4 , by R. N. Pederson and M. Ozawa in 1968 for a_5 and by Pederson and Schiffer in 1972 for a_6 .

De Branges's proof is obtained by way of two related conjectures. The first, due to M. S. Robertson in 1936, refers to 'odd' power series $b_1z + b_3z^3 + b_5z^5 + \dots$ univalent in the unit disk, and asserts the inequality $|b_1|^2 + \dots + |b_n|^2 \leq n|b_1|^2$. A simple argument shows that the Robertson conjecture implies the Bierberbach conjecture. In 1967 N. A. Lebedev and I. M. Milin proved an inequality about logarithmic coefficients of power series, which in 1971 led Milin to conjecture a further inequality which would imply the truth of the Robertson conjecture. It is this Milin conjecture that de Branges proves: the Robertson and Bierberbach conjectures follow immediately.

The method used by Löwner in his successful attack on a_4 is to introduce a partial differential equation whose solutions approximate any function univalent on the unit disk. This differential equation represents an expanding flow, and it is possible to push information along the flow — for example, estimates of the size of Taylor series coefficients. De Branges's proof introduces auxiliary functions τ_k to hold the desired information, each defined by a differential equation involving all previous $\tau_1, \dots, \tau_{k-1}$. If these functions can be shown to have certain useful properties, then the Milin inequality follows. However, the combinatorics of the sequence of τ s rapidly becomes very hard to manage. Walter Gautschi verified by computer that the desired properties hold for all values of n up to 25 (implying the truth of the Bierberbach conjecture for $n \leq 26$), and later noticed that the general result followed from a theorem of Askey and Gasper about Jacobi polynomials.

De Branges originally obtained his result as the climax of a 385-page typescript of the second edition of his book *Square Summable Power Series*, where it appears as a corollary to a general theory making heavy use of functional analysis. His announcement of a proof was greeted with some scepticism. De Branges had earlier claimed a proof of the Bierberbach conjecture that turned out to be fallacious, and this may have damaged his credibility.

The conjecture has a history of leading mathematicians astray. As Carl FitzGerald says in a survey (*Not. Am. Math. Soc.* 32, 2; 1985) of the conjecture and the circumstances surrounding its solution: "The Bierberbach conjecture is not difficult; I have proved it dozens of times". Acceptance came only after de Branges visited the Steklov Institute in 1984, when his proof was confirmed by the members

of the Leningrad Seminar in geometric function theory. E. G. Emel'ianov found a simplification avoiding the use of functional analysis which was examined by mathematicians in the West, who made some further simplifications and agreed that de Branges really had solved the problem. The proof has now been made very short and direct: one version takes only five pages (FitzGerald, C. and Pommerenko, Ch. *Trans. Am. Math. Soc.*, in the press). De Branges independently found a similar simplification.

So now we know that the Bierberbach conjecture has a short and simple proof, based on standard techniques in geometric function theory. Something very close to the successful assault must have been attempted by dozens, if not hundreds, of mathematicians. Why was the proof not found earlier? A likely reason is the subtlety of the problem. The precise approach that was successful is very delicate, and without a great deal of previous work, partial solutions and 'experimental' results, it is unlikely that anyone would stumble upon the particular combination of conditions needed to make the proof work. Another possibility is that most of the attempts were somewhat half-hearted, because nobody seriously thought that the standard approach would work. Mathematicians habitually talk of certain methods as being "worked out", in the mining sense that everything of value that can be extracted from them already has been. It is usually considered to be a waste of time to attack difficult problems by 'exhausted' methods. Yet, here it has suc-

ceeded. There are other mathematical cases. Dehn's lemma in topology and the complex version of the Hahn-Banach theorem baffled everybody for years but were eventually solved by methods that are so natural that the real puzzle is why the solutions had not been spotted immediately.

The answer may be related to the structure of mathematical proofs. As an analogy, consider a game of chess. Chess openings have been analysed by generations of Grand Masters in the hope of finding a novel line of attack and springing a trap on an opponent who finds himself in unfamiliar territory and under pressure of time. But there are so many possible variations in chess that a complete analysis of even up to a dozen or so moves is not feasible — there is still room for an original line of development only a little off the beaten track. Nimzovich caused his opponents many headaches by opening with a knight, a move so ridiculous that nobody had bothered to take it seriously. The 'opening moves' in a mathematical proof are far more varied than in chess. A line of argument may seem to be a poor opening simply because everybody who tried to play the game in that way followed a similar pattern of obvious but poor moves, missing a brilliant variation that would crack the game wide open. Certainly it is unwise to write off a plausible line of attack just because nobody has yet been able to make it work. □

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Olfactory transduction

Welcome whiff of biochemistry

from Gordon M. Shepherd

As a detector of molecules, the olfactory receptor neurone is impressive, combining high sensitivity with a broad and rapid response. Given these properties, one might assume that the mechanism whereby these neurones transduce odour molecules into nerve signals would have been the subject of intense investigation. On the contrary, neuroscientists have gravitated towards the more tractable receptor mechanisms, such as those at the neuromuscular junction and in photoreceptors, where the stimulus is well defined and the receptors more accessible. Happily, the situation is beginning to change, and the report from Doron Lancet's laboratory on page 255 of this issue¹, of the apparent involvement of an odourant-sensitive adenylate cyclase in the sensory response, is an important step forward in the biochemistry of olfactory transduction.

The experiments were carried out in the olfactory epithelium of the frog (see Fig. 1). The initial step in odour transduction is believed to take place in the olfactory

cilia. Lancet's group obtained a cilia-enriched cell-free membrane preparation by dissecting out the simple nasal sacs of frogs, exposing them to a brief calcium shock, and differentially centrifuging out a preparation containing over 80 per cent cilia membranes. The basal level of adenylate cyclase activity in this preparation was almost 15 times higher than in either the whole epithelium or whole brain, where activity is also high. The adenylate cyclase activity was enhanced by GTP and compounds such as forskolin, and inhibited by guanosine 5'-O-(2-thio) diphosphate (GDP β S), thus meeting some of the common criteria for the activation of the adenylate cyclase by a guanine nucleotide binding protein (G-protein). And ribosylation catalyzed by cholera toxin permitted the identification of a polypeptide with relative molecular mass (M_r) of 42,000, suggesting a stimulatory G-protein (G_s) within the general class of GTP-binding proteins (also called N proteins) activated by hormones and neuro-

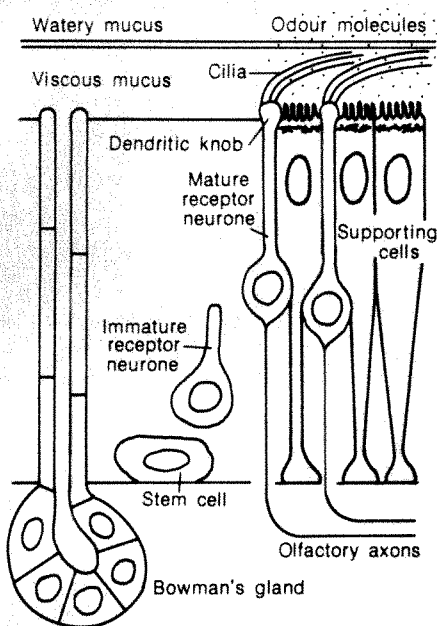


Fig. 1. Schematic diagram of the vertebrate olfactory epithelium. Mature receptor neurones arise during adult life by slow turnover of stem cells. Odour molecules are believed to act on receptor molecules (proteins?) in the membranes of the cilia and dendritic knob. Supporting cells have apical microvilli and are interconnected by a terminal web, as in other mucous membranes, thus limiting diffusion of absorbed molecules. They have glial-like properties and are believed to secrete the viscous mucus that bathes the surface. The thin watery mucus is thought to come from Bowman's glands.

transmitters.

If a G_s protein-adenylate cyclase system is involved in olfactory transduction, it should be affected by the presence of odour molecules. When the cilia preparation was exposed to a cocktail of four well-known odorant compounds, there was a GTP-dependent enhancement of the adenylate cyclase activity in a dose-dependent manner, at odour concentrations within the range of those used to stimulate receptor neurones in electrophysiological experiments.

From previous studies, it is known that field potential responses of the whole olfactory epithelium can be modulated by cyclic AMP analogues and phosphodiesterase inhibitors^{2,3}. The whole epithelium is a complex tissue (Fig. 1) in which the glia-like supporting cells and the glandular structures play undoubtedly important, but as yet undefined, roles. Because of these uncertainties and the limited data, the presence of a cyclic nucleotide cascade in the receptor neurones has been considered to be an open question⁴. The new study reduces the possibility of contamination by the other elements, and provides the clearest evidence yet of enzymatic processes related specifically to the sensory response.

The new data also fit nicely into an emerging outline for the sequence of steps that may be involved in olfactory transduction (Fig. 2a). Using this type of cilia-enriched preparation, Lancet's group has

previously reported the isolation of a 95K M_r glycoprotein that seems to be specific to the olfactory cilia⁵. They speculate that, like antibodies, this glycoprotein may have a constant and a variable region(s), the latter providing the diversity of sites required to account for the response of olfactory receptor neurones to a wide range of odour molecules⁶. Studies of other proteins that may be receptors are in progress elsewhere⁷.

By analogy with other second messenger systems, it seems a reasonable working hypothesis that binding of an olfactory molecule(s) induces an allosteric change in the receptor protein that causes activation of the G_s protein-adenylate cyclase cascade. One obvious advantage of this mechanism would be that the amplification built in to such an enzyme cascade would enhance the sensitivity of the receptor neurone; it could help to account for the ability of an individual neurone to be activated by single, or very few, molecules. Several types of receptor proteins might feed into the common enzyme pathway. Unfortunately, virtually nothing is known about the coupling of the adenylate cyclase to the presumed ionic channel(s) responsible for the receptor potential. This generates the impulse discharge that encodes the sensory response and transmits it to the brain.

The present report should provide a vigorous stimulus for further studies. Further biochemical characterization of the putative receptor protein and the G_s protein-adenylate cyclase pathway is urgently needed. Cloning of the genes for, and sequencing of, the olfactory receptor and G_s protein would then be not far from reach; the cloning of the gene for the α -subunit of transducin, the G protein in photoreceptors, has recently been reported⁸. Electrophysiological techniques have improved to the point where intracellular recordings can be routinely obtained from the olfactory receptor neurones, in spite of their very small size. Two possibilities deserve testing: first that the common action of cAMP causes phosphorylation of a channel protein and opening of ionic channels, resulting in the depolarizing receptor potential observed in response to odour stimulation; second, that there is a more direct effect on membrane conductance, similar to the direct action of cyclic GMP on membrane channels in rod photoreceptors^{9,10} (Fig. 2b). Whatever the mechanism, the extremely high sensitivity of olfactory neurones to injected currents suggests that small conductance increases, involving few channels, could produce large receptor potentials¹¹. Odour activation of single channels, and their control by adenylate cyclase, can be studied in patch-clamp recordings from cultured olfactory neurones¹² or in reconstituted membranes¹³.

Interest thus quickens. Perhaps there are other transducer mechanisms and pathways, such as through polyphos-

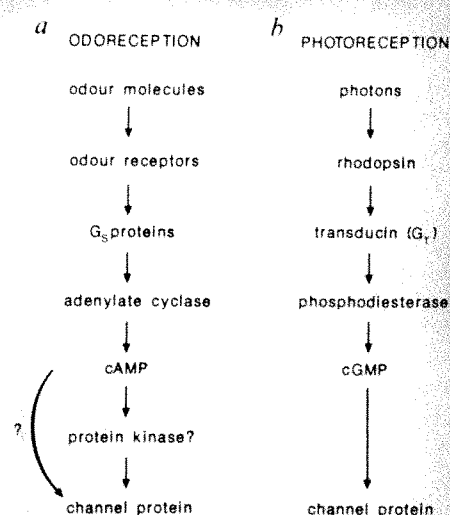


Fig. 2. a. Summary of proposed enzymatic cascade involved in olfactory reception⁶. b. The enzymatic cascade involved in visual reception^{9,14} for comparison.

phoinositol breakdown. The receptors could be modulated by neurotransmitters from autonomic fibres and nerve terminals, as well as by hormones carried through the rich vasculature of the epithelium. Perhaps the mechanisms of olfactory reception will turn out to be similar to those of the immune system, as noted above. There are intriguing similarities; for instance, as well as recognizing a variety of molecules, olfactory neurones are continuously generated throughout adult life from precursor cells (Fig. 1). If the analogy with the immune system finds support, we may eventually characterize olfactory transduction as involving 'odogens' which stimulate 'odobodies', leading to changes in activity of the intracellular enzymatic pathways discussed above and, perhaps, also to the release of extracellular signals that communicate with the surrounding supporting cells and regulate the differentiation of new receptor neurones from the precursor cells. Undoubtedly, there are pleasant surprises awaiting us right under our noses. □

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Optics

Reflections on closed loops

from David K. Lynch

REFLECTION and refraction at the surface of gently rippled water produces images containing a variety of closed curves, the shapes of which depend on the spatial, or angular, distribution of the incident light field. The loops usually represent the distorted boundaries of high contrast features in the scene. In these columns¹ recently Thomas Gold discussed the formation of closed loops in reflections of linear objects such as the mast of a sailing boat, but point sources and objects extended in two dimensions also form closed-loop images. Here, I compare the loops resulting from reflection and diffraction of all three types of source.

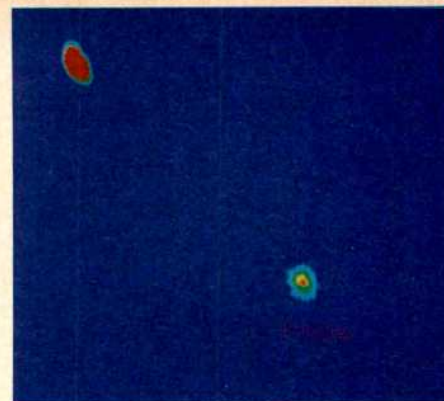
All real sources are extended to some degree but sources that approximate to a point, such as the Sun and Moon, create a myriad of rapidly formed loops (Fig. 1), usually in the shape of distorted ovals^{2,3}. Because the water contains many surfaces inclined at precisely the correct angle to reflect light to the observer, the casual observer sees the loops — which form almost too rapidly to follow — coalesce to create the familiar glitter patch^{4,5}. These are readily seen in any body of water illu-

minated by a point source high overhead, such as bath tubs, swimming pools and coffee cups. The statistics of such reflections have been discussed by Longuet-Higgins², but the details of loop formation have not been well studied.

Theoretical studies of point sources show that there is not a one-to-one correspondence between the source and its image: that is, the solutions are not unique. For example, when the local radius of curvature of the water is twice the distance between the observer and the surface, the image of a point source is spread into a two-dimensional patch of light.

For linear sources of light such as masts, the reflected structure depends on the angle of the line of sight and the orientation of the source^{1,3}. Since a vertical mast separates two extended, nearly identical featureless regions, the blue sky seen inside a loop comes from one side of the mast and the blue region surrounding the loop comes from the other, a distinction that cannot be made for point sources.

With extended sources, such as mountains and clouds, the closed curves form landpools and skypools (W.C. Livingston,



First sight of Halley

CCD image of comet P/Halley (1982i) obtained on the 18 April 1985 with the 4.5-m Multiple Mirror Telescope of the Fred L. Whipple Observatory (MMTO). The integration time was 10 minutes and the seeing FWHM was 0.8 arc seconds. The comet image extends 5 arc seconds in the sunward direction with a brightness V of 18.7. The object at the top left is a binary star whose images have been smeared in following the comet's motion. The contoured bands of the cometary image indicate that the coma has been resolved. The image was digitally processed by K. Hege of MMTO. The first spectra from the coma are discussed on page 241.

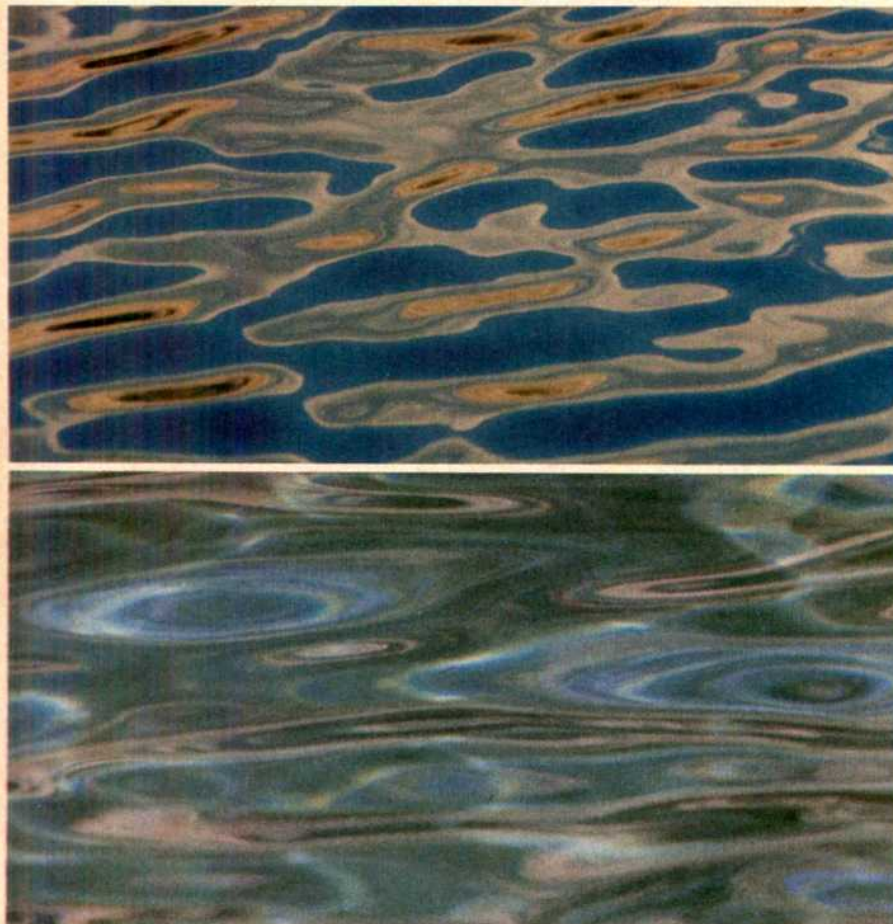


Fig.2 (top) Skypools and landpools in a reflection of an extended source.
Fig.4 (bottom) Refracted images of the sandy bottom of a lake.

personal communication), regions of reflected blue sky light surrounded by darker regions from the landscape and vice-versa (Fig.2). Except for the distortion, landscape re-imaging is often extremely accurate on water in terms of surface detail. The reflected blue sky seems almost white at the boundary of the skypool. This is simply the reflection of the true brightness and colour distribution of the sky made readily visible because many tens of degrees of vertical sky are compressed into a small angle. However, skypools are not distorted images of the entire sky because only a small part of the sky is actually re-imaged; the upper and lower parts of the skypool originate from the same part of the horizon and approximate to mirror images.

Refraction can also render objects of all three categories into closed curves. The most common examples can be seen on the bottom of a swimming pool. High contrast point-like sources (most commonly the Sun) appear as loops, analogous to those formed by reflected points. Such linear sources, distorted by refraction, become the familiar bright webs on the bottom when light is focused by the wavy surface of the water⁶ (Fig.3). Because of dispersion, these loops also show brilliant colours. Extended sources, such as a bright rocky or sandy bottom, also display closed contours when viewed at low incidence. In these images the phenomenon is developed to its most fascinating complexity (Fig.4).

Much of the structure in both reflected and refracted distortions depends on the

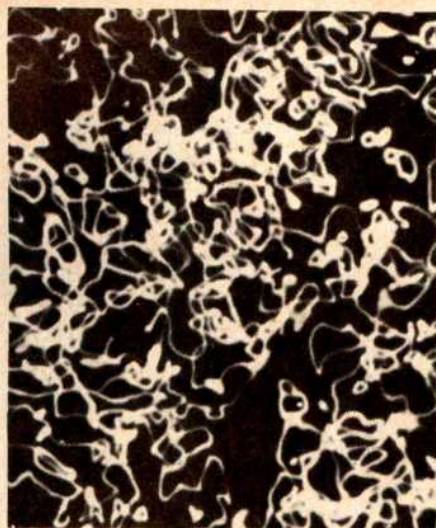


Fig.1 Reflection of a point source: the moon.
Fig.3 Refracted image of a linear source from the bottom of a swimming pool.



duration of the observation and the spatial distribution of the source. If photographs of point sources reimaged by the water are taken with shutter speeds far shorter than the period of the waves, the image usually shows many sharp loops, together with partial loops caught as the shutter opens or closes in the middle of a trajectory. Exposures much longer than the wave period result in overlapping images. For a point source against a dark background, overlaps are simple but photographs of an extended source create patterns of colours and shapes that rival the finest impressionist art. □

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Geomagnetic field

Volcanic record of reversal

from Jean-Pierre Valet, Carlo Laj and Piotr Tucholka

ON PAGE 230 of this issue Michel Prévot and his colleagues report the most detailed palaeomagnetic record of a reversal in the Earth's geomagnetic field to have been obtained from lava sequences¹. Because the type of rock in which the record is preserved determines certain characteristics of the record itself, it is of particular interest to compare some characteristics of the Steens Mountain record of Prévot *et al.* with similarly detailed records obtained from sediments.

Important data on the morphology of the transitional geomagnetic field have been obtained in the past decade from palaeomagnetic records of reversals. The first big step was the recognition that the transitional field is not dipolar². K.A. Hoffman then attempted to recognize an average field configuration³; in some cases there is an indication of axial symmetry⁴ but non-axisymmetrical components are present in most detailed results, both for single transitions⁵ and for sequences of reversals⁶. Although many attempts have been made to determine the harmonic content of the transitional field^{7,8}, further clarification of this problem needs records of transitions from the Southern Hemisphere.

The records have generally been obtained from sediments or from lava sequences. Magnetization of sediments is associated with the continuous process of sedimentation so that the resulting signal depends on the deposition rate and on the time necessary for the locking in of the remanence. If the acquisition process is very prolonged, the result can be a heavy smoothing of the signal. Laboratory studies^{9,10} have shown that magnetization is locked in at a depth of about 10–20 cm in the sediment, a value which has recently been confirmed by a novel method¹¹. Moreover, the pattern of the record itself can be used to determine the importance of this effect. Despite these apparent drawbacks, it is only sediments that can provide continuous, multiple records of the same transition from widely separated localities as well as records of sequences of reversals.

The magnetization in lavas is acquired in a very short time while the lavas are cooling down in the ambient field. Therefore, a sequence of lava flows provides a succession of instantaneous pictures of the transitional field and so is very sensitive to the rapid directional changes of the field on a very short timescale. Determinations of the absolute palaeofield intensity from lava, unlike sediments, are particularly reliable but, unfortunately, although the sequence of flows can be well established, it is impossible to determine the exact tim-

ing of the eruptions. Also, records from lavas provide neither verification of results from multiple profiles nor sequences of records from consecutive reversals.

The transition recorded at Steens Mountain in Oregon is characterized by the succession of two distinct phases, the first being a complete flip from normal to reverse of the vector, while the second is a normal–transitional–normal rebound. Most of Prévot *et al.*'s discussion is given over to the field variations associated with the second phase. During this period, the directional changes are mostly scattered far away from the meridional plane and the virtual geomagnetic poles describe large longitudinal variations. There are also two important directional jumps, which the authors interpret as geomagnetic impulses associated with global changes of the field with time constants of 1–2 years. Note that these impulses occur while the field configuration is away from axial symmetry. Following the suggestion of the authors that such impulses reflect an increase of the turbulence within the Earth's core, the results also suggest that the whole field configuration is affected by this increase. The authors note that time constants of the same order of magnitude as the impulses have been observed for the 1969 secular variation jerk. It is thus important to connect this observation with a recent theoretical prediction by J.L. Le Mouél¹² that some of the observed features of the secular variations should persist during a transition.

The two phases of the transition are separated by an episode of normal directions and pretransitional intensity values, which is interpreted by Prévot *et al.* as the temporary re-establishment of the dipole field configuration. In view of the thickness of the recording zone, they argue that the first episode lasted half the time of the second, so that the whole record would correspond to a single reversal. But this argument rests on the assumption that the volcanic activity was regular and uniform which is, at least, questionable.

Alternatively, therefore, it can be suggested that the polarity change was completed at the end of the first phase and that the second phase is a record of an excursion that is quite separate from the major reversal. Of course, this interpretation implies very different characteristics of the transitional field, closer to those that are commonly observed in sediments. In particular, the directional changes are much more regular and the resulting directional path is highly confined in a narrow longitudinal band, suggesting a degree of organization of the transitional field. We note also that the first fast

impulse lies well within the meridional plane. Fast changes within transition paths are also apparent in sedimentary records but are often disregarded as possible disturbances of sedimentary or other origin. Perhaps in the light of the Steens Mountain results they will merit new consideration.

If the second episode reflects an excursion of the geomagnetic field that is unrelated to the polarity reversal, the Steens Mountain record provides evidence that the field behaviour associated with these two phenomena is different. The core processes during reversals and excursions would, at least in this case, not be intimately related. In particular, the hypothesis of K.A. Hoffman¹⁴ that palaeomagnetic excursions are aborted reversals would not be valid.

Although Prévot *et al.* certainly have written an important chapter, the history of palaeomagnetic records of transitions is not finished. Our knowledge of the transition field should be increased by multiple and detailed records of the same reversals. Thus, records from sedimentary sequences offer the most promising per-

spectives. In particular, very important results can be expected from records with very high sedimentation rates. This should allow time resolution in a range similar to that which is possible for volcanic rocks and so should lead to a reconciliation of the sedimentary and volcanic records. □

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Climate modelling

The climate as natural oscillator

from Gerald R. North

THE Earth's climate exhibits great variability at timescales centred at 100, 41 and 20 kyr, as shown convincingly by J. Hays *et al.* (*Science* **134**, 1121; 1976) using data from marine sediments. As these oscillations happen to be the dominant periods of the variations in the Earth's orbital elements (eccentricity, obliquity and perihelion at summer solstice), they have stimulated a revival of the astronomical theory of the Quaternary glaciations (see *Milankovitch and Climate* eds Berger, A. *et al.* Reidel, Dordrecht, 1984).

Steady-state atmospheric climate models show remarkably little sensitivity to orbital changes, leading to a major crisis in climate theory over the past decade. An interesting line of reasoning has been pursued by Barry Saltzman and colleagues (for example, *J. atmos. Sci.* **41**, 3380; 1984). To avoid the issue of sensitivity, Saltzman suggests that the system is a natural nonlinear oscillator with a period of about 100 kyr. The tiny orbital forcing merely acts as a pacemaker providing a phase and precise frequency to lock with.

Although it is premature to call the Saltzman theory conclusive, its elegance and generality make it essential reading for ice-age buffs. The model is highly idealized, keeping track of only a few important dependent variables. It consists of a set of first order (in time) differential equations — the prognostic equations — for the perturbed variables: land-ice volume, sea-ice volume and deep ocean

temperature. The differential equations are coupled through simple polynomial forms up to the third degree in powers of the variables. The solution for equilibrium (all variables vanishing) exists, but it is unstable to infinitesimal perturbations from equilibrium for reasonable values of the coefficients in the system. The free solution state is quickly drawn away from the origin to a limit cycle with a period of about 100 kyr.

The dominant physical process in the model is the sea ice cover. When it is large, the planet has a large albedo leading to an overall cooling and further growth of ice volume by expansion of the freezing line towards the equator. On the other hand, large areas of ice-covered ocean inhibit evaporation and therefore starve the glaciers. Other feedbacks, such as inhibition of heat transport towards the pole under the sea ice and the subsequent buildup of deep ocean temperature, act in the same general manner. If the time constants for these processes are different enough they can lead to oscillations with rather asymmetrical growth and decay time characteristics. The longest time constant introduced in the problem as a parameter is about 7 kyr in the thermal inertia of the deep ocean. The nonlinear dynamics of the system translates this relatively short timescale into the much longer natural oscillation period. The sharp termination of ice volume in the data is one of the unanswered questions about the 100-kyr

cycles. This sawtooth time series, consisting of slow ice growth and abrupt terminations, seems to be a natural property of the Saltzman model, arising from the differences in time characteristics of the individual components. Parameter sensitivity studies show that the essential character of the oscillation is unchanged by small departures from the nominal values.

Although the Saltzman model is attractive because of its logical consistency and systematic formulation, it is not alone in the arena of contenders for ice-age glory. M. Ghil and colleagues (*J. geophys. Res.* **88**, 5167; 1983) suggest an oscillator model based on a short natural period (about 10 kyr). When driven by the Milankovitch frequencies, the solutions exhibit combination tones, one of which is near the 100-kyr period. In their theory, R. Peltier and J. Hyde (*J. atmos. Sci.*, in the press) emphasize feedbacks associated with the bedrock response. The latter theory does not exhibit free undamped oscillations, but it does seem to have a strong response at the 100-kyr period when driven by a forcing frequency near a harmonic of this natural period, say 20 kyr.

There has never been a shortage of ice-age theories, because many of the component processes of the system cannot yet be modelled. The result is a group of schemes replete with free parameters and fudge factors — the Saltzman theory has about 10 such factors and others are comparably endowed. The biggest challenge facing the ice-age system theorist seems to be finding a risk that a model can run in the face of hard evidence as opposed to fitting the lone time series of ice volume. In terms of fitting the ice volume time series it is hard to surpass the phenomenological model of J. Imbrie and J. Z. Imbrie (*Science* **207**, 943; 1980), purely because of its simplicity. The model included one time constant for ice volume growth, one for its decay and a scale parameter for the forcing strength. The result was a rather good fit to the sawtooth time series. The main task now faced by modellers is to construct theories that derive these parameter values from more fundamental principles.

The Achilles heel of simplified theories modelling time behaviour of ice volume is their sensitivity to the parameterization of snowfall over the ice sheets. This is especially grim as the most detailed general circulation models of the atmosphere seem unable to compute the precipitation rates adequately, particularly near the poles — just where it counts in an ice-age theory. The next decade may lead to a better understanding of such precipitation, producing a breakthrough comparable to geological evidence in terms of eliminating classes of ice-age theories. Meanwhile, modellers can explore the vastness of parameter space unconstrained by cruel realities. □

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Simpler DNA sequence representations

SIR—The method of DNA visualization presented by Hamori^{1,2} admits of a simpler representation which may be more generally useful. In Hamori's technique, each of the four nucleotides is represented by a vector in three-dimensional space having a characteristic, but variable, orientation. DNA structure then may be viewed from any chosen perspective in two-dimensional plots on computer graphical monitors. Different aspects of structure are examined by varying the geometric relationships among the four elemental vectors.

I suggest that a particular, canonical choice of vectors has certain advantages, both for exploring structure and for comparing sequences. Figure 1 illustrates an intronic sequence based on this approach, wherein successive guanine (G) residues are represented by unit vectors in the positive x-axis direction, complementary cytosine (C) residues correspond to negative x-axis unit vectors, and thymine (T) and adenine (A) residues correspond to unit vectors in the positive and negative y-axis directions respectively.

With this choice, all sequences can be represented in two dimensions in a unique manner, and differences in sequence structure become immediately apparent. In Fig. 2, which compares two actin genes, the regions of divergence of the exonic sequences are clear.

Furthermore, this method of embedding DNA sequences in a two-dimensional metric space permits the consistent application of several standard concepts based on distance measures. Two are worth mentioning. Plots of cumulative Manhattan distances from a site of origin (for example, the initiation codon) against position number can be useful in pinpoint-

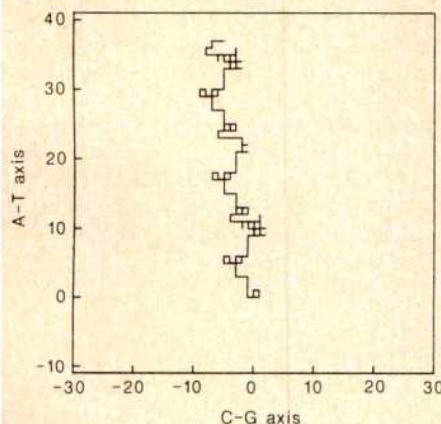


Fig. 1 Graphical representation of the 186-base pair (bp) nucleotide sequence of human BK papovavirus BK enhancer³, showing three 12-bp repeats. The sequence begins at the bottom, at coordinate position [0,0], and reads (5' to 3') towards the top. Successive G, C, T and A nucleotides are depicted as coordinate shifts of one unit in the +x, -x, +y and -y directions, respectively.

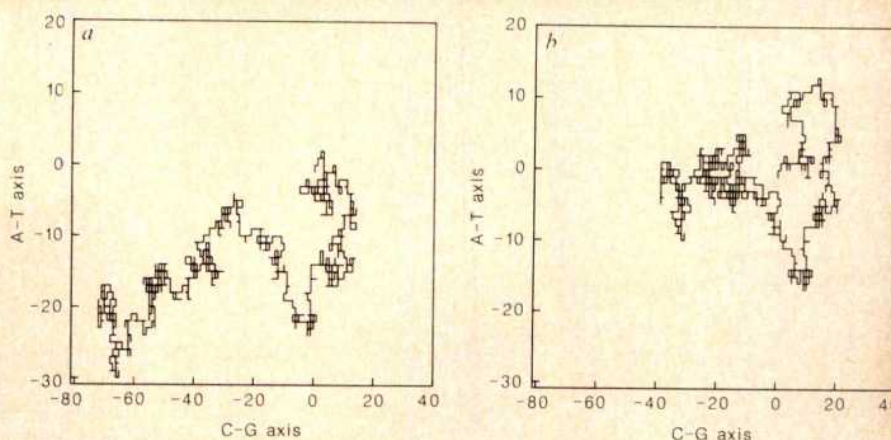


Fig. 2 Nucleotide sequence representations of two actin genes from *Drosophila melanogaster* (Canton S strain). The single intron has been removed from each, leaving a 1,131-bp exonic sequence. Data were obtained from the GenBank library. a, Cytological locus 79B; b, cytological locus 88F.

ing similarities in structure of sequences coding for the same type of product; this ideally should be done after alignment for maximum sequence homology.

Finally, the general properties of classes of sequences (for example, actin genes from different groups of organisms, different types of immunoreceptors) can be examined by calculating the fractal dimension, in this space, of each sequence. This is simply the ratio of the logarithm of the total Manhattan path length to the logarithm of the distance of the end-

point of the sequence from its origin: $\log [n(A) + n(G) + n(T) + n(C)] / \log [n(G) / n(C) + |n(T) - n(A)|]$, where $n(X)$ is the number of occurrences of nucleotide X in the sequence.

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Similarity of vaccinia 28K, v-erb-B and EGF receptors

SIR—Vaccinia virus, a member of the Poxviridae, has a large, double-stranded DNA (about 187,000 base pairs) and replicates in the host-cell cytoplasm. The genome contains more than 100 genes, some of which probably code for the enzymes involved in the viral transcription process. Recently, similarities were found between the sequences of the vaccinia 19K protein, EGF, transforming growth factor type 1, and several other mammalian proteins¹⁻³.

Using the FASTP rapid similarity search⁴ and other programs of the Protein Identification Resource system, we have now found unusual sequence similarity (Figs 1,2) between the vaccinia virus 28K proteins a major late protein of unknown function, synthesized after DNA replication and the carboxyl ends of the v-erb-B transforming protein of avian erythroblastosis virus⁵ and, to a lesser extent, the related human epidermal growth factor (EGF) receptor precursor⁷.

When we used the ALIGN program to evaluate these similarities, the 28K protein and a portion (345-584) of the carboxy-terminal domain of the v-erb-B protein produced an alignment score of 4.9 s.d. (mutation data scoring matrix with 6 added to each term, penalty of 6 for a break in either sequence, 300 random

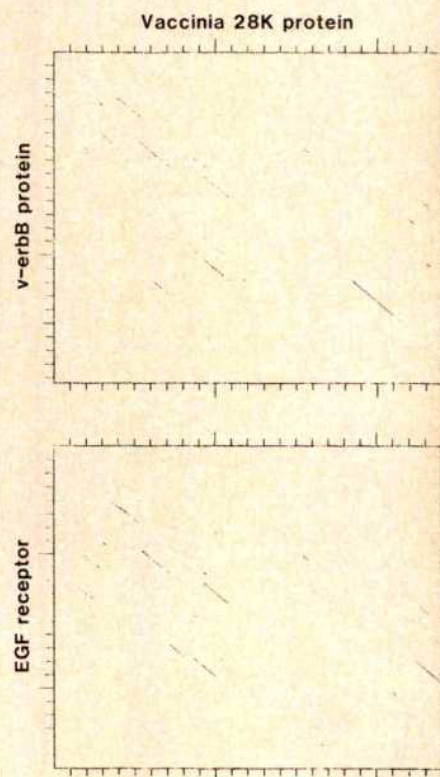


Fig. 1 Graphic matrix plots of the vaccinia 28K protein, the v-erb-B protein (350-604), and the EGF receptor precursor (920-1,170). Each point represents a score of 20 or more over a span of 25 amino acids using the mutation data scoring matrix.

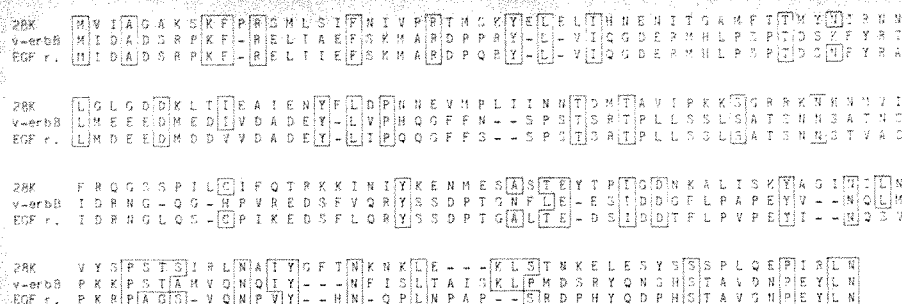


Fig. 2. Alignment of vaccinia 28K protein (28-229), v-erb-B protein (372-562), and EGF receptor precursor (952-1,140). Identities between the 28K protein and either of the other two are boxed.

permutations). A score of 7.2 s.d. was obtained if portions of the 28K protein (28-229) and the v-erb-B (372-562) were compared. Comparison with a portion (952-1,140) of the cytoplasmic domain of the precursor EGF receptor produced a score of 5.6 s.d. Because of the exhaustive nature of the FASTP search, values in this range indicate a very interesting similarity that is likely but not certain to have biological significance⁴.

Such similarity can result from common ancestry or from convergent evolution of proteins with similar structures or functions. For example, it may be advantageous for the virus to mimic certain host proteins. It remains to be seen if more vaccinia proteins have sequences similar to host proteins. Perhaps the relationship between vaccinia virus and its host cell will become clearer when the entire genome of this virus is completely sequenced and more host sequences are available.

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Did Burton synthesize diamonds in 1905?

SIR—In a letter to *Nature* of 24 August 1905¹, C. V. Burton, a prominent physicist, claimed to have synthesized diamond at ambient pressure by an unusual method. It may seem odd that the first response to this communication should appear some 80 years later, but the strange fact is that, for some inexplicable reason, this disclosure was lost to the literature.

Although a brief abstract appeared in *Science Abstracts*, it was not abstracted by any of the three chemical abstracting journals available at that time nor has any chemical treatise concerning carbon referred to it. Despite the voluminous literature relating to synthetic diamonds, not one author has referred to Burton's work. By chance, one of us (F. S.), found a reference to Burton's letter in a book on gem

stones by W. Goodchild², published in 1908 when the memory of Burton's disclosure was still fresh in the author's mind. Burton gave only a skeleton outline of his procedure and promised a further communication. However, a meticulous search has not disclosed such a communication.

Although Burton claimed to make diamond in the region of temperature and pressure in which graphite was the stable form of carbon, there is no reason why he should not have been successful in obtaining diamond in a metastable state. His method was to dissolve carbon in a lead-calcium alloy in which carbon is more soluble than in lead alone, then supersaturate the lead by oxidizing the calcium with steam at a "dull red heat" thus removing it from the alloy. The carbon precipitated in the form of diamond. Ostwald's law of successive reactions, also now lost from physical chemistry textbooks, provides for a metastable phase to appear before the stable phase, and at 500°C, the conversion of diamond to graphite is exceedingly slow. The data Burton gave on the crystals he obtained, their octahedral form, the refractive index, and the resistance to chemical attack are consistent with his claim to have made microscopic diamonds.

We have attempted to repeat Burton's work. In the absence of any experimental details, it is difficult to be sure that we followed his procedure exactly, and we had some difficulty incorporating the carbon in the alloy but we successfully solved that. We decomposed the alloy with steam at 550°C, our estimate of a dull red heat. The grey crust was treated with acid and washed well to remove all lead and calcium salts. We obtained a black powder in which were embedded many transparent crystals which scintillated with considerable fire in reflected light. They were at most a few micrometres in size. Burton claimed that he made only diamonds and no graphite. We obtained considerable amounts of black powder which may or may not have been graphite which made it impossible to separate the tiny crystals for precise identification. The crystals had a high refractive index and the powder scratched glass. X-ray powder diffraction showed a strong peak at 0.208 nm which is the strongest peak for diamond, but only weak for graphite. Strangely, the

strongest peak for graphite was absent, though two other peaks at 0.161 nm and 0.148 nm were still present. The strongest peak was an unidentified one at 0.251 nm. On the basis of this information, we cannot claim unequivocally that diamond was produced, but combined with the other properties, there is a strong presumption that diamonds were made and, therefore, Burton had synthesized diamonds in 1905, half a century before General Electric had made them in the stable region of high temperature and pressure. He deserves recognition in the Hall of Fame which includes Moissan and Hannay.

Thanks are expressed to the National Science Foundation for providing a grant to enable this work to be done, to L. W. Zelazny for his help in providing the X-ray data, and to F. D. Bloss for assistance with the refractive index.

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Misapprehension over Alzheimer's disease

SIR—Stanfield *et al.* make the *en passant* observation that degeneration of the neurones of the nucleus basalis of Meynert "are the likely cause of Alzheimer's disease"¹. This is not so. However, it is a widely held misapprehension. Other nuclei besides the nucleus basalis are similarly afflicted with tangles and show similar cell loss in Alzheimer's disease^{2,4}: lesions to the nucleus basalis area in animals produce few of the clinical and none of the pathological symptoms of Alzheimer's disease. The article commonly quoted⁵ as support for the cholinergic hypothesis has the beautifully ambiguous title "Alzheimer's disease: a disorder of cortical cholinergic innervation". This title is correct in only one of its senses.

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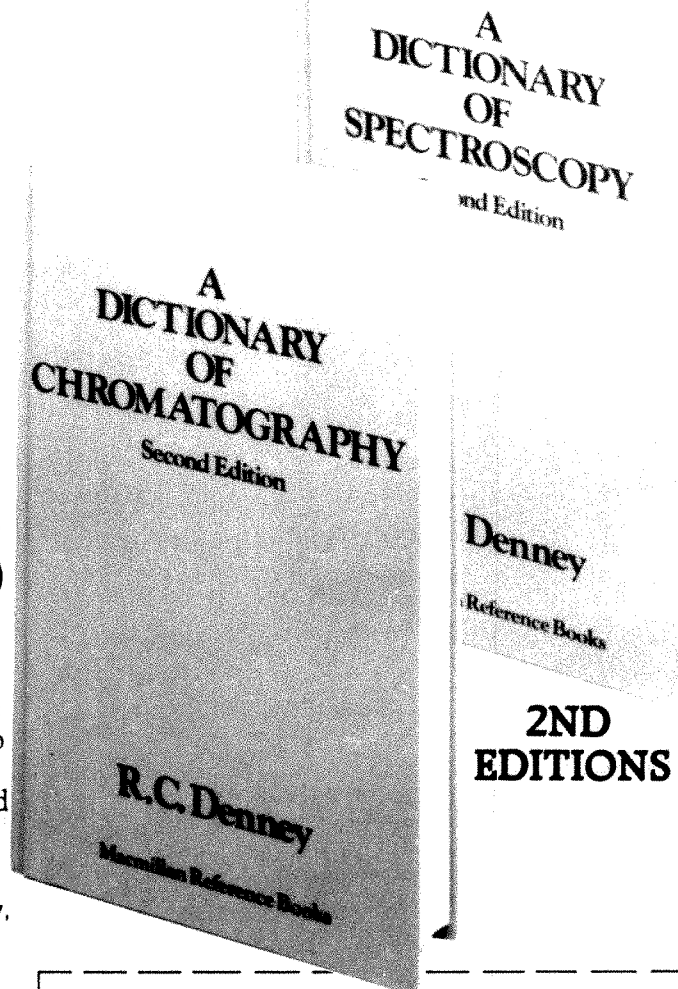
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D. M. MacKay

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THE popular image of science has undergone some curious reversals of fortune over the past century. Fifty years ago, the rabble-rousers of the *avant garde* preached the gospel of "scientific humanism", according to which science was the ultimate answer to all the problems besetting mankind, including those traditionally the province of religion. In more recent years the fashionable trend has been to turn upon science as itself the origin, rather than the remedy, of the ills of our age.

In times like these the corrective of a broader historical view is much needed, and Colin Russell's book seems admirably designed to meet the need. As Professor of the History of Science and Technology at the Open University he has both the knowledge and the communicative skills to bring alive the ways in which religious and scientific ideas have interacted over the centuries to shape our conception of the natural world. Following in the footsteps of such pioneers as R. Hooykaas (whose classic *Religion and the Rise of Modern Science* is frequently cited), he shows how from early Greek times onward people's ideas of God, or the gods, have coloured their attitude to nature and helped or hindered their efforts to understand it. In the end, refuting popular ideas to the contrary, he is able to marshal "powerful historical evidence of a massive mutual debt between Christianity and science":

Conflict there has certainly been, but always for reasons that are peripheral to the real issues with which science and Christianity are concerned. Only in that limited, even localized, sense has there been anything like even an "uneasy truce". Desperate attempts to evacuate the Christian faith of its essential content, so as not to offend the susceptibilities of "scientific man", are not merely misguided but leave most scientists profoundly unimpressed [p. 252].

More negative historical phases are not neglected. The mixed reception given to Copernicanism is carefully analysed, and the popular notion that the heliocentric model was seen by contemporaries as a "demotion of man" is cogently questioned. Rhetoric's long-lost treatise designed to demonstrate Copernicus's orthodoxy, recently re-discovered by Hooykaas, takes a line rather similar to Kepler's in pleading that Holy Scripture (with its poetic references to the "fixity" of the Earth) was not intended to be used as a textbook of science. One gets the impres-

sion that if Galileo had been a little less aggressively tactless, the Roman Church might have been coaxed to recognize in time (rather than two centuries after the fiasco of his trial) the theological innocence of his proposals.

The part played by Puritanism in the rise of modern science has been much debated since R. K. Merton highlighted it. Russell gives a fair picture of the controversy, concluding that

underlying the bewildering variety of parties associated with science was a common core of biblical allegiance common to Puritanism, to the wider Calvinism and indeed to Protestantism as a whole. Of the resonance between that allegiance and the growth of science there can be no possible doubt [pp. 83-84].

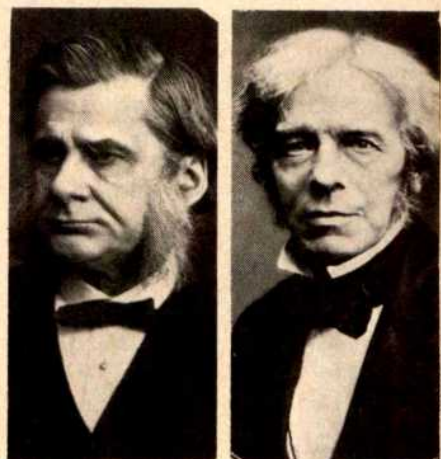
Moreover

[People] are surprised that the tight-lipped, joyless exponents of an iron religious creed could ever unbend to consider such frivolities as scientific experiments, let alone have the wit to understand them. Yet that is only because "Puritan" and "puritanical" are given a totally unhistoric meaning today. The early Puritans, like Bunyan, did take their faith seriously, but they laughed and made music and love like anyone else, and they, more than their contemporaries, delighted in the world of nature [p.82].

An illuminating section on the Darwinian controversy draws on recent scholarship, especially on the work of J. R. Moore, to reassess the parts played by religious (and anti-religious) convictions among both supporters and antagonists of evolutionary theory. Despite their hostility to the established Church, T. H. Huxley and his Victorian friends were curiously soft-centred in their attitude to nature — almost as if they hoped to find in "her" the God they had lost. Huxley's view that "living nature is not a mechanism but a poem" typifies the Romantic strain in much agnostic thought of the day. Russell gives some fascinating glimpses of the pseudo-religious fervour with which the new and optimistic "scientific" creed was followed. It would seem (though he does not directly say so) that the aggressively anti-religious X-club founded in 1864, together with such vocal protagonists of "Victorian scientific naturalism" as J. W. Draper and A. D. White, showed in their vehemence and their disregard for awkwardly relevant facts quite as much extremism as we nowadays deplore in anti-evolutionary fundamentalism. Conversely, Russell makes clear that a num-

ber of convinced Christians were from the outset enthusiastic (perhaps too uncritically so) in support of Darwin's biological theory (as distinct from the atheistic metaphysics that borrowed its name and prestige). The fact that men of the stature of Joule, Maxwell, Kelvin and Faraday remained quietly convinced believers is also cited against any idea that the leaders of Victorian scientific thought, as a body, felt themselves to be at war with religion.

The concept of science as a potential boon to man, of which Francis Bacon made so much, was evidently alive already in the thirteenth century. It is all the more curious that, despite the declared endorsement of it by the fledgling Royal Society, this idea for so long bore so little



Victorian protagonists — T. H. Huxley (left) and Michael Faraday.

practical fruit in technology. Painstaking improvements in craftsmanship brought rich dividends. Advances in the design of chronometers, for example, revolutionized the art of navigation. But according to Russell it is not until the middle of the nineteenth century that we find scientific insight as such contributing significantly to technological advance; and even then, as the story of thermodynamics shows, the traffic was by no means all one-way.

Readers who wonder how some modern bishops' views on miracles fit into the historical picture will find Colin Russell well prepared to answer; and chapters on the impact of quantum theory on theology, and of the Christian doctrine of stewardship on ecological thinking, ensure that contemporary issues are not overlooked. Though Professor Russell writes as a Christian, he maintains a judicial posture and a scholarly regard for documentation that makes his book specially suitable for students and those concerned to see the record straight. Appetizing as well as authoritative, it should be required reading for teachers of science as well as of religion, whether in or out of the pulpit. □

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Cloning cookbook for the laboratory

Kevin Struhl

A Practical Guide to Molecular Cloning. By Bernard Perbal. Wiley: 1984. Pp.554. Hbk £68.85, \$59.50; pbk £39.90, \$34.50.

TEN years ago, molecular cloning was an art that was practised only in a few Californian laboratories. Now, with everyone and his brother involved in recombinant DNA manipulations, the field has exploded into a vast and complex technology that is far beyond the ability of an individual to learn and remember completely. Furthermore, because these techniques have become invaluable in almost all fields of biological science, it is frequently the case that those who want to use them are not trained as molecular biologists. In short, there is a great need for a cookbook, which contains a reasonably complete collection of recipes that are easy to follow and up to date.

Three years ago Cold Spring Harbor Laboratory published such a collection, *Molecular Cloning: A Laboratory Manual* by Maniatis, Fritsch and Sambrook. This book is omnipresent in molecular biology laboratories and is utilized to the point where it is frequently referred to as "The Bible". Although experts usually use shortened versions of the procedures, the methods are clearly described and they do work. Indeed, the only shortcoming of this manual is its age. It does not contain anything on the production of monoclonal antibodies and related methodologies, now standard tools of the trade, and the section on expression vectors for the controlled production of gene products is weak and out-dated. An updated version is clearly necessary, and the authors are, in fact, preparing a new edition.

What does *A Practical Guide to Molecular Cloning* offer that the earlier book lacks? Unfortunately, the answer is not much. The most practical addition is that of DNA sequencing using single-stranded DNA vectors and primer extension with di-deoxy nucleotide precursors. However, most commercial suppliers enclose an equally detailed protocol on purchase of the reagents. The author also describes a number of techniques for the purification

of large quantities of DNA. These methods are seldom used and the descriptions read suspiciously like the promotional literature from the companies that sell the products.

Given the need to include newer techniques, Perbal devotes an inordinate number of pages to unnecessary matters. More than 20 per cent of the book is spent on lists of restriction endonucleases and vectors; such information, although useful, is easily found in the catalogues of commercial enzyme suppliers. Thirty-three pages are devoted to a "theoretical study of the fraction of a long-chain DNA that can be incorporated in a recombinant DNA partial-digest library" and the representation of DNA sequences in such libraries. I don't know anyone who uses this information on a practical basis; moreover, the few qualitative aspects worth knowing are buried among pages of mathematical formulae.

Members of my laboratory found Per-

bal's book much harder to use than its competitor. One problem is that the book is bound in a conventional fashion, as compared to the earlier manual which comes as a spiral notebook. This seemingly trivial difference is actually of practical importance because Maniatis *et al.* lies flat and is thus easy to read on a laboratory bench, its natural habitat. Another problem is that the experimental protocols appear in large print, whereas explanatory paragraphs appear in small print. This is visually uncomfortable, and the large print for the protocols coupled with the small page size means that many techniques are spread out over a number of pages.

Thus it seems to me that the Maniatis *et al.* manual is still the best around, and the revised version should be even better. □

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Genesis of man

Harry V. Merrick

African Archaeology. By David W. Phillipson. Cambridge University Press: 1985. Pp.234. Hbk £25, \$39.50; pbk £9.50, \$14.95.

OVER the past two decades the pace of prehistoric archaeological research in Africa has accelerated enormously. Although recent work frequently has not produced as visually spectacular discoveries as in other parts of the world, it has been of the first order of importance in elucidating earlier human behaviour, especially during the Stone Age. Far from being a culturally stagnant cul-de-sac during the course of human evolution, Africa has often displayed a technological precociousness that would have astounded prehistorians 20 years ago.

In this book Dr Phillipson has brought together the new findings with earlier research to produce a very readable, relatively non-technical survey of the current state of knowledge of African prehistory. It is both timely and urgently needed; the last comparable synthesis is now 15 years old and has been largely superseded by recent events.

African Archaeology is very much a traditional, narrative account of the main stages of human development in Africa, emphasizing subsistence patterns and general ways of life. Each stage is presented in a series of regional summaries, accompanied by illustrations of the principal sites and representative tool and pottery types. Fortunately, much of the nomenclature and the intricacies of stone tool classification, which have dominated chronological and cultural reconstructions in African prehistory, are

neatly bypassed using Grahame Clark's scheme of technological modes to describe levels of technological complexity without temporal connotations. Nonetheless, details of chronology and technology still loom large for the first two million years of prehistory recounted in the first third of the volume. However, for the past several millennia, and especially for the Sub-Saharan region, Phillipson combines the findings of archaeology, including results from his own considerable field work, with linguistic and historical evidence and oral tradition to produce a much fuller view of emerging African societies in each part of the continent. This is an approach which also encourages appreciation of some of the environmental, economic and social factors underlying the cultural diversity of modern African peoples.

The bold attempt to encompass over two million years of a continent's prehistory in a single volume has understandably necessitated a synoptic treatment of most of the more controversial issues in African prehistory. While the superficial treatment of these problems may be trivial in the broader context of a continent-wide survey, regrettably it represents a missed opportunity to convey the excitement in African archaeological research today and the increasing inclination towards problem-orientated research. That apart, the book is impressively comprehensive, well-referenced and concise. It will be of particular interest to a broad range of Africanists and archaeologists, and of value as an introductory textbook for courses which cover African prehistory and history. □

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New in paperback

- *Quarks, Gluons and Lattices* by M. Creutz. Publisher is Cambridge University Press, price is £7.95, \$12.95. For review see *Nature* 309, 290 (1984).
- *From Folk Psychology to Cognitive Science: The Case Against Belief* by Stephen Stich. Publisher is MIT Press, price is \$8.95, £8.95. For review see *Nature* 309, 815 (1984).
- *Ontology and Phylogeny* by Stephen Jay Gould. Publisher is the Belknap Press of Harvard University Press and the price is \$8.95, £8.25.

Of the highest order

W.C. McGrew & J.R. Anderson

The Natural History of the Primates. By J.R. and P.H. Napier. *British Museum (Natural History)*: 1985. Pp.200. £15. To be published in the United States later this year by MIT press.

Primates in Nature. By Alison F. Richard. W.H. Freeman: 1985. Pp.558. Hbk \$27.95, £32.95; pbk \$17.95, £13.50.

IN 1967, the Napiers' *A Handbook of Living Primates* appeared and immediately became the standard reference source for primatologists. Now the same authors have written another, slimmer reference work. There are several similarities between the two books: in each the bulk of the text is made up of potted profiles of primates arranged by genus, although the proportion given over to these has dropped in the new book, and some of the photographs and figures are the same as before. The differences, however, suggest that *The Natural History of the Primates* is aimed at a wider, more popular audience. New features are the inclusion of colour plates, a more spacious, double-columned lay-out and use of British as well as metric measures, while there is minimal citation of sources and only a short reference list. The writing is lively and clear, and the last chapter on human evolution packs a lot into only ten pages.

But the book does not fare so well in its stated role as a textbook. The content is sometimes repetitive (for example, three times, on pp. 19, 31 and 53, we are told that marmosets have claws and not nails) and is also somewhat old-fashioned in places. Key advances in the sociobiology and behavioural ecology of primates made over the past 15 years are rarely mentioned. Thus, for example, predators on primates are described as "beneficial" in helping to maintain the prey species as a "balanced viable population". Overall, this is a book which will better serve the keen amateur than the student or professional.

By contrast *Primates in Nature* is remarkable for its breadth of approach and the depth of ecological information provided; it deserves to become a cornerstone in the primatologist's library. One of the author's aims was to treat primates "as mammals rather than as our closest living relatives", that is to illuminate ecological generalities as well as features particular to primates, as in the adaptations of certain langurs and macaques to the problems of living in seasonally harsh temperate zones. Such comparisons do not dominate the book, however. Apart from a discussion of social organization in African hunting dogs and lions, non-primates take a back seat in Chapters 4-10 which concentrate on what is and is not

known about primates: as individuals (feeding, diet, reproduction), populations (demography), groups (social organization) and communities (interspecific relationships). The final chapter compares the position of primates and non-primates in ecological communities.

Dietary constituents and their distribution in plants are described lucidly in Chapter 4, followed by accounts of the feeding regimes of various species and of some of the corresponding morphological adaptations, notably dental and gastrointestinal. Some may feel that vertebrate prey are dismissed rather casually, for example in view of known seasonal and individual variations in meat-eating in chimpanzees. The same could be said for insectivory: while specialist insectivores



Demidov's Bush-baby taken from *Monkeys and Apes* by Gotthard Berger and recently published by Arco. The price is \$24.95.

get their fair share of attention, insect-eating by non-specialists is passed over more quickly.

The author has adopted a case-history approach in describing aspects of primate ecology, illustrating a number of topics such as reproductive parameters, demographic patterns, social organization and so on with examples drawn from a range of species. This provides a broad perspective on primates as an order, and it is good to see prosimians and callitrichids given equal place alongside their larger and better-known counterparts. Anyone studying a field-site with more than one species of primate will benefit from reading Chapter 10 on sympatry, competition and the niche.

Primates in Nature does not include many data on social behaviour, for instance on grooming rates and on mother-offspring interaction. But it does contain solid background reading, a glossary of ecological terms and a wealth of ideas for preparing a primate field study, all in highly readable style. □

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Got that loving feeling

Michael Shepherd

Aphrodisiacs: The Science and the Myth. By Peter V. Taberner. Croom Helm/University of Pennsylvania Press: 1985. Pp.275. £19.95, \$22.50.

WHAT IS an aphrodisiac? According to Peter Taberner, it is, strictly speaking, an exciter of lust; more generally, "anything which, by any means, increases the capacity for sexual enjoyment". With this definition he has given himself a broad canvas on which to sketch a readable, fact-filled review that roams over the wilder shores of sexuality.

As a professional pharmacologist the author is well qualified to discuss the properties and adverse effects of both the relatively small number of sexual stimulants in the medical pharmacopoeia and of the drugs of abuse which are used for their supposedly aphrodisiac properties. These constitute, however, no more than a small proportion of some 500 animal, vegetable and mineral substances listed in one of the appendices which have been or are being used as putative aphrodisiacs and to which reference is made in the text.

As its title indicates, the book is concerned with myth as well as science, with witchcraft and wormwood as well as bromocriptine and brain stimulation, with anthropology and medical history as well as the endocrinology and neurophysiology of sexual behaviour. The attainment of heightened sexual satisfaction ranks with gold, immortality and happiness as one of mankind's long-standing chimeras; as Havelock Ellis pointed out 80 years ago, "the early history of this subject is more or less inextricably commingled with folklore practices of magical origin". It still is, and Taberner has legitimately dipped into the rich fields of ancient tradition, magic, herbalism and quackery to provide many telling illustrations of human hopes and credulity, though pornography is unaccountably absent from the index.

A final chapter is devoted to aphrodisiacs of the future, and deals with such possible forerunners of a brave new world as pheromones, aromatherapy and cerebral stimulation. All a long way from the Act Two duet of Donizetti's *L'Elisir d'Amore*, in which the heroine Arina and the mountebank Dulcamara sing of the rival claims of love philtres and female charms, agreeing in the end that no potion is a substitute for genuine feeling. But perhaps they are singing of something other than Aphrodite. □

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Lots of GUTs

D.H. Perkins

Fifth Workshop on Grand Unification. Edited by Kyungsik Kang, Herbert Fried and Paul Frampton. *World Scientific/Wiley*: 1984. Pp. 538. \$59.50, £51.75.

THE gravitational force between two protons is 10^{-38} of the electrical force. Where does such an odd number come from? Understanding the interrelation between the fundamental interactions of nature and their possible unification has always been a primary goal of the physicist. Regular conferences and workshops are devoted to the subject, the one in 1984 at Brown University being the fifth in a present series. This book contains the record of the meeting.

The first, and arguably the greatest intellectual leap was taken by Newton 300 years ago, when he proposed that the same universal gravitational force that made the apple fall also held the planets in orbit about the Sun. The next was the unification of electricity and magnetism by Maxwell in 1869. The third was the proposed unification of the weak and electromagnetic interactions by Salam, Weinberg and Glashow in the late 1960s which predicted the existence of the massive bosons W and Z, discovered at CERN two years ago.

Grand unification theories (GUTs) are a proposed further stage, originally identified with unification of the strong quark-quark force with the electroweak, but more recently taken to include gravity as well — thus encompassing all the known interactions in nature.

Maxwell's theory of electromagnetism was quickly verified by the detection of electromagnetic waves by Hertz, and the electroweak theory by the discovery of neutral weak currents in 1973 and the W and Z ten years later. However, despite early promise — the correct prediction of the value of the electroweak mixing angle — there is as yet hardly any experimental support for GUTs. The theory occurs in several versions, all of them making several predictions, none of them so far borne out by experience.

One of the early predictions was that of proton decay. This was not a new idea. Sakharov, in the context of the big bang model of the Universe, had pointed out in 1966 that the mechanism required to generate the huge preponderance of matter over antimatter in the Universe must also be responsible for proton decay at some level. Since 1973, GUTs had predicted a lifetime of around 10^{30} – 10^{33} yr, that is within experimental reach of huge (1 kiloton) detectors.

Papers in the present volume describe results of four experiments carried out deep underground in three continents. Their main achievement has been to ex-

clude one version of GUT: the lower limit on the lifetime for decay of a proton to a positron plus a neutral pion exceeds 10^{32} yr, more than an order of magnitude longer than the prediction. Needless to say, the other versions of GUTs make less definite predictions and so are harder to exclude. Yet all the experiments found candidates for other decay modes at 5–10 per cent of the total event rate, which is dominated by the atmospheric neutrino background. Finding out whether (or how) protons decay is going to be a long experimental struggle, and the question is hardly likely to be resolved by the tenth or even the fifteenth workshop in the series.

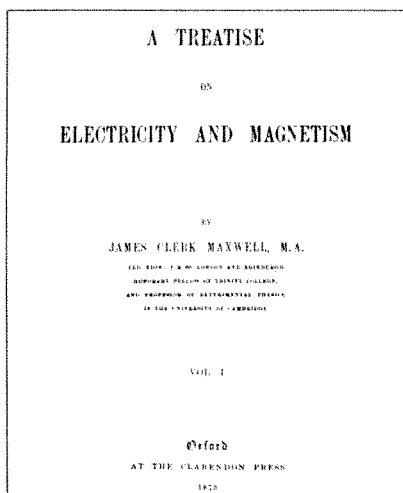
Another prediction of some versions of GUT is that leptons are not conserved and

plus re-heat" version of the big bang is that the density of monopoles can be reduced to an acceptable number (one per galaxy, for example). Again, and just as in the case of neutrino oscillations, early possible signals have not been repeated and the most recent and sensitive experiments, seeking to detect monopoles through their ionization or currents induced in superconducting loops, find no evidence for their existence. Astrophysical limits on monopole flux, although inherently less reliable, set even more stringent flux limits.

Grand unified theories incorporating supersymmetry, in which it is postulated that all bosons are mirrored by fermion partners and vice versa, have become fashionable in recent years. None of this host of supersymmetric partners has so far been detected. In $p\bar{p}$ collider data at CERN over the past year, a few mysterious events (monojets) with large missing energy might be consistent with production of gluinos (the fermion counterparts of the gluons mediating the interquark force) — or equally with something else. Latest rumours are that even this possible signal may be vanishing.

Finally, theorists have proposed supergravity models, some of them based on the ideas of Kaluza and Klein of more than half a century ago. These start off with Einstein's theory in many dimensions, all but four of which are compacted and unobservable but which can be interpreted as the other fields (electromagnetic, strong and so on).

This volume gives a blow-by-blow account of the present status of the experimental and theoretical work in this most fundamental area of science. The hard core of practical evidence gives strong support for the old-hat "standard model" — of quark and lepton constituents, of the gauge models of the strong and electroweak interactions — and that is a solid achievement. The standard model is however somewhat ugly and theoretically unattractive because we cannot understand the huge range of particle masses, from less than 10 eV for neutrinos, through 10^{11} eV for the W and Z bosons, to 10^{28} eV for the Planck mass. So the theorists have gone off into what appears to many experimentalists to be esoteric wildernesses which have a beauty and symmetry of their own, and one of which might even provide the final answer. The main problem is that experiments in particle physics are very expensive and complex, and take five to ten years from first inception to final results. Those running now were inspired by the now out-dated theory of a decade ago. In short, we have lots of ideas and questions but, so far, hardly a real clue. □



1873 — Maxwell publishes his work on the unification of electricity and magnetism.

that the different flavours of neutrino may transform one into the other. Historically, this was one possible way of accounting for the fact that the flux of electron-neutrinos from the Sun is a factor of three smaller than expected. Numerous attempts over the years to detect this flavour mixing — resulting in oscillations, for example in the electron-neutrino flux as a function of distance from a nuclear reactor — have all failed. With the exception of a single Russian experiment on tritium β -decay, the interpretation of which is open to question, all results to date are consistent with zero mass neutrinos and zero mixing. Four articles in the volume are devoted to recent results on double β -decay (setting upper limits on the neutrino mass) and on oscillation experiments at reactors and accelerators.

A third major prediction of GUTs is the existence of magnetic monopoles. These were originally invented by Dirac over 50 years ago, as a way of "explaining" quantization of electric charge. In GUTs however, the charge quantization follows from the construction of the theory, and the result is that massive elementary monopoles, of mass 10^{16} GeV or 10^{-8} g, absolutely have to exist. In the original big bang model of the Universe, enormous numbers of monopoles would be predicted. A free bonus of the "inflationary

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Intensity-dependent quasi-periodic oscillations in the X-ray flux of GX5-1

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The X-ray flux of the bright galactic bulge source GX5-1 shows intensity-dependent quasi-periodic oscillations between ~20 and ~40 Hz, appearing as a broad peak in the power spectrum whose centroid frequency, width and integrated excess power strongly depend on the source intensity. The strength and steepness of low-frequency noise present in the power spectra below 15 Hz also depend on the source intensity. No evidence is found for coherent X-ray pulsations between 0.5 and 2,000 Hz. We discuss possible mechanisms to explain these new phenomena.

NEUTRON stars in low-mass X-ray binaries have long been suspected to have been spun up to periods in the millisecond range. Several searches for such short rotation periods in low-mass X-ray binaries have been made but none has been successful (refs 1-4 and J. Fleischman, S. Rappaport, D. Strahs and W. Lewin, personal communication). We have renewed the search using the medium energy detectors of the X-ray observatory Exosat. One of the first sources we observed was the bright bulge source GX5-1, in which we found no periodic pulsations but, unexpectedly, we discovered quasi-periodic oscillations. These oscillations are the subject of this report; some preliminary results have already been reported⁵.

Observations

We observed GX5-1 (4U 1758-25) with the medium-energy detector⁶ (ME) and the gas scintillation proportional counter⁷ on Exosat. The observations started on 18 September 1984 11:50 UT and lasted for ~8 h. We shall discuss 1-18-keV data obtained with the Ar/CO₂-filled proportional counters of the medium-energy detector; the counters were co-aligned with a total effective area of 1,500 cm². The data were obtained in 2-s blocks, each containing 8,192 samples (time resolution ~0.25 ms). Within each of the 1,623 blocks, the data are continuous with a negligible 'dead time' of ~1% and between two consecutive data blocks there is a gap of 15 s. The data contain no spectral information.

Figure 1 shows the counting rate as a function of time. The data include an approximately constant background of ~80 c.p.s. The sharp drop near $t = 17,000$ s is caused by a change ('trim') in the satellite pointing direction. The reason for this trim was the high counting rate, at which detector damage was feared. Before the trim, the collimator efficiency was 93%, whereas afterwards it was 65%.

For the average spectral shape, as measured with the gas scintillation proportional counter, 1 c.p.s. from the ME (pre-trim collimator efficiency) corresponds to $\sim 9.2 \times 10^{-12}$ erg cm⁻² s⁻¹ (1-18 keV), or ~ 0.3 μ Jy (2-11 keV). The source flux (~ 700 - $\sim 1,100$ μ Jy) and its variability on a timescale of hours are similar to those encountered previously⁸⁻¹². All count rates described here have been reduced to the pre-trim collimator efficiency.

Analysis and results

To investigate the variability of GX5-1 on timescales between 0.5 ms and 2 s, we estimated the power spectrum by calculating (via a fast Fourier transform (FFT) algorithm) the Fourier amplitudes of the average-subtracted signal in each 2-s block

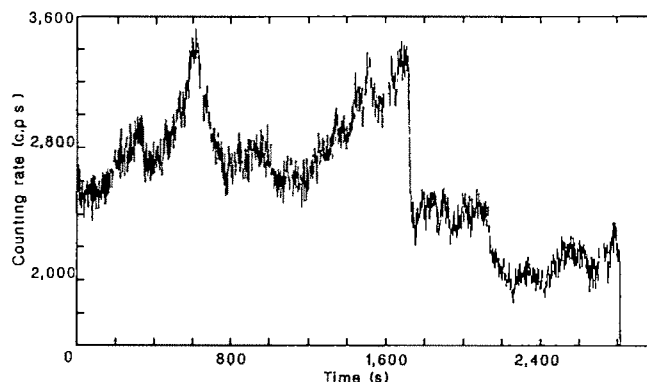


Fig. 1 Average counting rates in 2-s time intervals. Zero time corresponds to 18 September 1984, 11:53:35 UT. The sharp drop near $\sim 17,000$ s is the result of a change in the pointing direction of the satellite (see text).

separately. The power spectra of single data blocks are too noisy to allow meaningful study. However, the average of these power spectra clearly shows three components: (1) low-frequency noise below 15 Hz (the power increases towards lower frequencies); (2) a strong broad peak with a centroid frequency near 30 Hz; and (3) a flat spectrum above 100 Hz, consistent with Poisson noise.

No evidence was found for coherent pulsations in the X-ray flux. The 99% confidence upper limit to the pulsed fraction of coherent pulsations in the 50-400-Hz range is 0.3%; in the 400-2,000-Hz range it varies between 0.6 and 2.5%, depending on frequency. In calculating these values, we followed the procedures described by Leahy *et al.*³ and took into account possible Doppler shifts resulting from orbital motion of the source and the satellite.

Power density estimates, using conventional FFT techniques, may be in error because of low-frequency 'leakage', if a steep low-frequency ('red') noise component is present¹³. The power-law slope of the red noise in our data is considerably below the critical value of 2, above which problems may occur. Low-frequency leakage is therefore not expected to affect our analysis. This was confirmed by tests on artificial red-noise data and by repeating the analysis of the 2-s blocks after 'detrending' each of them by subtracting a low-order polynomial.

The properties of the broad peak in the power spectrum (centroid frequency, width and integrated excess power) and the low-frequency noise (slope, and integrated excess power) strongly depend on source intensity. In Fig. 2a, b we show the

observed power spectra as a function of time and source intensity, respectively. The broad peaks in the power spectra are seen in Fig. 2a as a dark 'ribbon'; the low-frequency noise is visible at the bottom. The centroid frequency ν_c is clearly correlated with the source intensity (Fig. 2a). Also, both the quasi-periodic oscillations and the low-frequency noise diminish at high source intensity. The dependence of ν_c on source intensity is approximately linear (Fig. 2b). This dependence is not affected by the $\sim 30\%$ drop in counting rate caused by the 'trim' near $t = 17,000$ s (Fig. 1). A power-law dependence, however, is also consistent with the data.

The fact that ν_c depends on source intensity and not on the counting rate in the instrument convinced us that the peak in the power spectrum is related to a phenomenon in GX5-1 and is not an instrumental effect or an artefact of the analysis. In this context we mention that background data obtained immediately before and after our observation with the same time resolution, and observations of several other sources of similar brightness, did not show a peak in the power spectrum.

As far as we can tell at this stage of the analysis, all variable properties of the power spectra depend on the source intensity only. To describe the power spectra quantitatively, we performed least-squares-fits to the averages of power spectra selected from six source-intensity intervals. As a fitting function we used

$$P(\nu) = A + B e^{-\gamma\nu} + C[(\nu - \nu_c)^2 + (\lambda/2)^2]^{-1} \quad (1)$$

where P is the power density and B and C are normalization constants. The first term on the right-hand side (a constant) describes the white-noise component. In our adopted normalization³ one expects $A = 2$ for poissonian noise. The low-frequency noise is well described by an exponential function (second term in equation (1)). A power law does not adequately fit the data.

We used a Lorentz profile with a full-width at half maximum, λ , and centroid frequency ν_c (third term in equation (1)) to describe the peak. We also tried to fit the peak with a gaussian profile; this did not give better power-law fits to the low-frequency data. (The width and integrated excess power of the peak are then 20–30% lower.)

The fits of expression (1) to the averaged power spectra (first 512 frequencies) have a reduced χ^2 between 1.03 and 1.17 for 506 degrees of freedom. The variance of the power estimates was taken as $4/N$, N being the number of power spectra averaged³. Examples of the fits are shown in Figs 3 and 4. For decreasing source intensity, ν_c decreases, the peak becomes narrower and the low-frequency noise component steepens.

Figure 5 shows plots of the numerical results (listed in Table 1). The centroid frequency of the peak (Fig. 5a) shows a strong, approximately linear correlation with source intensity. The peak width (Fig. 5b) and the steepness of the low-frequency noise spectrum (Fig. 5c) show an approximately monotonic dependence on the source intensity. The integrated excess power of both the peak (Fig. 5d) and the low-frequency noise (Fig. 5e) show a maximum, roughly halfway in the observed intensity range.

The width and integrated excess power of the peak are 30–50% lower than shown in Fig. 5 (b, d) if we choose a power-law description of the low-frequency noise (both for a lorentzian and a gaussian peak profile). The centroid frequency is very insensitive to changes in the fitting function.

In principle, the broad peaks in the averaged power spectra could result from the averaging out of very narrow peaks which occur at different frequencies in the power spectra of the individual 2-s data blocks. However, the peak remains broad when the data are analysed in intervals as short as ~ 100 s.

We shall assume that the averaged power spectra are representative of those of the individual 2-s blocks. The results can then be interpreted as resulting from a signal that shows quasi-periodic oscillations of which the typical period decreases from 50 to 25 ms and the coherence time (defined as the e folding time of autocorrelation function) from 75 to 25 ms when the source intensity increases by $\sim 30\%$. The signal also contains low-frequency noise to which the contribution of slower vari-

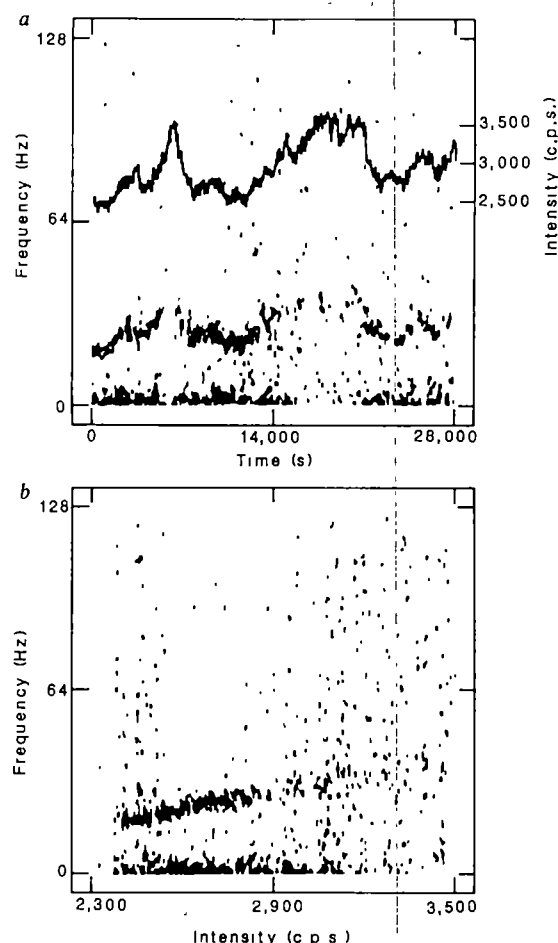


Fig. 2 a, Display of the temporal variation of the power spectra. The frequency is in the vertical direction (left-hand scale). A vertical cross-section of the display is a grey-scale coded representation of a power spectrum. The darker the shade the higher the power it represents. To conserve the quality of the picture, the original display of 512×512 pixels has been smoothed with a gaussian profile with $\sigma = 1.3$ pixels. The variable broad peak in the power spectra is visible as a dark 'ribbon'; the low-frequency noise is visible at the bottom. Also shown is the intensity of GX5-1 (right-hand scale). The peak frequency depends strongly on source intensity. Also, notice that both the quasi-periodic oscillations and the low-frequency noise diminish at high source intensity. b, Display (see a) of the power spectra as a function of source intensity (reduced to pre-trim collimator efficiency). The peak frequency is approximately linearly related to source intensity.

ations becomes weaker for increasing source intensity and of which the strength (integrated excess power) depends on the source intensity in a way very similar to that of the peak.

The noise process that underlies the quasi-periodic character of these oscillations is not strongly constrained by our present analysis; the counting rates are too low to allow us to follow individual wave trains. It could be the result of (see, for example, ref. 14): (1) a superposition of randomly excited damped harmonic oscillators; (2) random phase variations of a periodic wave train; (3) the superposition of closely spaced periodic oscillations whose frequencies are distributed over a finite interval. It is also conceivable that the broad peak is the result of a dependence of the peak frequency on the X-ray photon energy, which is smeared out because of the absence of energy resolution in our data.

Discussion

Intensity-dependent quasi-periodic oscillations, such as those reported here, have not been observed previously (millisecond X-ray oscillations lasting of the order of 10 s have been reported^{2,15} which are probably unrelated). The variations in frequency of the oscillations clearly exclude the possibility that

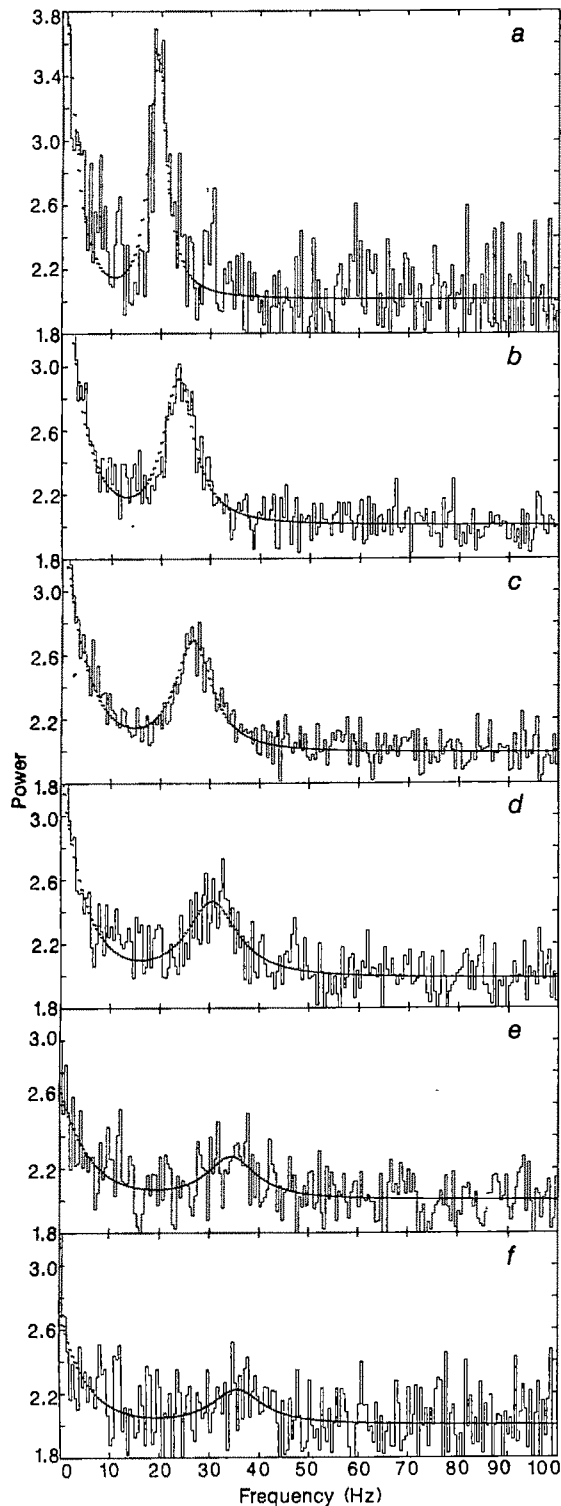


Fig. 3 Linear displays of average power spectra of GX5-1 in six different source-intensity intervals. *a*, 2,277–2,486; *b*, 2,486–2,695; *c*, 2,695–2,904; *d*, 2,904–3,113; *e*, 3,113–3,322; *f*, 3,322–3,531 (c.p.s.). The average has been taken over the power spectra of individual 2-s intervals (see text). Fits of equation (1) to the data are also shown. Notice the change of centroid frequency and width of the peak as a function of source intensity.

they represent the rotation period of the compact object.

Quasi-periodic oscillations on timescales of the order of tens of seconds are a common phenomenon in cataclysmic variables^{14,16,17}. Of particular interest are the optical oscillations seen during some dwarf-nova outbursts which show a positive correlation between frequency and (optical) flux^{14,18,19}. Apart from the $\sim 10^3$ times longer period and the different energy band, these oscillations differ from those in GX5-1 by: (1) the generally one to three orders of magnitude larger coherence; (2) the

positive correlation between coherence time and source intensity¹⁹ (negative in GX5-1, see Figs 3, 4, 5*b*); and (3) the much weaker dependence of frequency on optical flux.

Models proposed for these dwarf-nova oscillations include inhomogeneities rotating with the inner accretion disk^{20,21}, or with the white dwarf envelope²²; disk oscillations^{23,24}; oscillations in the atmosphere of the white dwarf^{25,26}; and various instabilities in the accretion flow (for example 27–29; for reviews see refs 14, 18). We examine some of these models in the context of our new findings for GX5-1, assuming that the compact object is a neutron star; in the case of cataclysmic variables the compact object is a white dwarf.

The dependence of the frequency of the oscillations on the source intensity in GX5-1 is strong ($d \log \nu_c / d \log I \sim 2$). The accretion-flow instability models of Langer²⁸ and Livio²⁹ can be excluded on this ground alone. The model of King²², which involves magnetic loops anchored in a differentially rotating envelope of the compact object, predicts the correct sense for the correlation of intensity with frequency and coherence of the oscillations, but does not make quantitative predictions.

Non-radial *g*- or *r*-mode oscillations of the neutron star envelope could have periods in the range observed by us^{30,31}. Proposed excitation mechanisms for these oscillations are thermonuclear flashes³² (see also ref. 33) and sudden accretion events³⁴. However, X-ray bursts have never been observed from GX5-1. It is unclear whether, with a mass-accretion rate varying by $\sim 30\%$, the second mechanism can be considered viable.

In Bath's²⁰ model for dwarf-nova oscillations, the oscillations originate from inhomogeneously distributed matter ('blobs') orbiting the compact object with the Kepler frequency at some preferred radius, in particular that of the magnetosphere, r_m . In the case of spherical accretion, r_m is given by^{35,36}

$$r_m = (2.9 \times 10^8 \text{ cm}) \mu_{30}^{4/7} m^{1/7} R_6^{-2/7} L_{37}^{-2/7} \quad (2)$$

Here μ_{30} is the magnetic moment of the compact object in units of 10^{30} G cm^3 , m its mass in units of solar masses, R_6 its radius in units of 10^6 cm and L_{37} the X-ray luminosity in units of $10^{37} \text{ erg s}^{-1}$. In our further discussion we assume $m = R_6 = 1$.

The keplerian frequency ν_K at the magnetospheric radius depends on the X-ray luminosity according to

$$\nu_K = (0.37 \text{ Hz}) L_{37}^{3/7} \mu_{30}^{-6/7} \quad (3)$$

With $\nu_K \sim 30 \text{ Hz}$ and $L_{37} \sim 10$, one infers $\mu_{30} \sim 2 \times 10^{-2}$ or, equivalently, a surface magnetic field strength $B_0 \sim 2 \times 10^{10} \text{ G}$ and $r_m \sim 160 \text{ km}$. The relation between the frequency of the quasi-periodic oscillations and the luminosity for GX5-1 is qualitatively as predicted by equation (3). However, as mentioned above, the observed value for the exponent is about five times the predicted value of $3/7$, and this may indicate that this model²⁰ is not applicable here. Alternatively, disk oscillations^{23,24} could occur near, for example, the magnetospheric radius. The periods of disk oscillations are of the order of the Kepler periods³⁷; the oscillations would then occur at a radius of $\sim 10^2 \text{ km}$. The quasi-periodic oscillations in the X-ray flux could, for example, be produced by intermittent occultations of the X-ray source by the orbiting blobs or by the oscillating disk.

Warner²¹ suggested that the observed frequency, ν_c , of the dwarf nova oscillations is the beat frequency of the (keplerian) disk frequency, ν_K , at the magnetospheric radius and the rotation frequency, ν_s , of the white dwarf. Alpar and Shaham^{38,39} suggest the same idea to explain the oscillations in GX5-1. For the case of the neutron star this leads to

$$\nu_c = (0.37 \text{ Hz}) \mu_{30}^{-6/7} L_{37}^{3/7} - \nu_s \quad (4)$$

The dependence of ν_c on luminosity can then be made stronger at will by a proper choice of the value of ν_s . A fit of relation (4) to our data (dashed curve in Fig. 5*a*) yields $(\nu_s)^{-1} = 10.5 \pm 0.4 \text{ ms}$ and $B_0 = (6.3 \pm 0.3) \times 10^9 (D/10 \text{ kpc}) \text{ G}$; here D is the source distance. The magnetospheric radius is $\sim 60 \text{ km}$.

The derived magnetic field strength depends strongly on whether the blobs are seen at the magnetospheric radius, r_m (equation (2)), or at some other radius. According to Ghosh and Lamb⁴⁰, for disk accretion, the appropriate magnetospheric

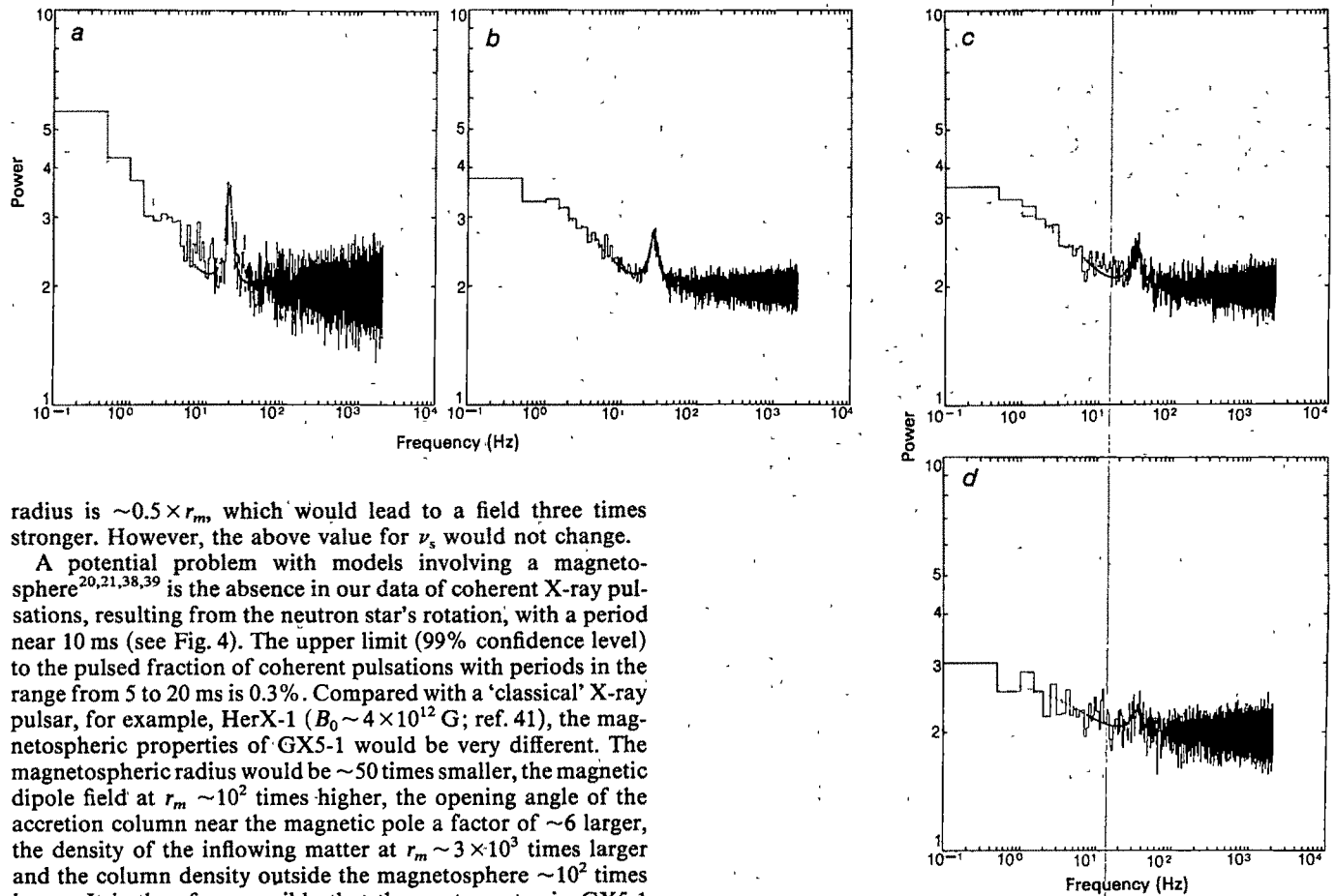


Fig. 4 Logarithmic displays of average power spectra in four different source intensity intervals (see Fig. 3 legend). *a*, 2,277–2,486; *b*, 2,695–2,904; *c*, 2,904–3,113; *d*, 3,113–3,322 (c.p.s.). The dependence of the low-frequency noise on source intensity is seen more clearly than in Fig. 3.

radius is $\sim 0.5 \times r_m$, which would lead to a field three times stronger. However, the above value for ν_s would not change.

A potential problem with models involving a magnetosphere^{20,21,38,39} is the absence in our data of coherent X-ray pulsations, resulting from the neutron star's rotation, with a period near 10 ms (see Fig. 4). The upper limit (99% confidence level) to the pulsed fraction of coherent pulsations with periods in the range from 5 to 20 ms is 0.3%. Compared with a 'classical' X-ray pulsar, for example, HerX-1 ($B_0 \sim 4 \times 10^{12}$ G; ref. 41), the magnetospheric properties of GX5-1 would be very different. The magnetospheric radius would be ~ 50 times smaller, the magnetic dipole field at $r_m \sim 10^2$ times higher, the opening angle of the accretion column near the magnetic pole a factor of ~ 6 larger, the density of the inflowing matter at $r_m \sim 3 \times 10^3$ times larger and the column density outside the magnetosphere $\sim 10^2$ times larger. It is therefore possible that the neutron star in GX5-1 cannot be seen directly because it is obscured by plasma. It is also conceivable that a special geometry of 'X-ray beams' may hide the signature of the neutron star spin, or that the magnetic and rotation axes are co-aligned.

Despite the fact that the beat-frequency model^{21,38,39} has been introduced in a rather *ad-hoc* fashion to account for the observed relation between frequency and intensity, the inferred rotation period and magnetic-field strength of the neutron star fit well³⁸ into an existing evolutionary framework for the galactic bulge sources^{42,43}, which links these objects to the 6-ms radio pulsar⁴⁴ PSR1953+29.

In this evolutionary model^{42,43}, neutron stars in bright galactic bulge sources are spun up to millisecond rotation periods because of accretion from an evolved companion, and the X-ray

sources evolve into wide binary radio pulsars^{45–47}. (That a spun-up neutron star with a relatively weak magnetic field, in a binary, may later on show up as a fast radio pulsar was first suggested by Smarr and Blandford⁴⁸ and further elucidated in refs 49–53.) The rotation period of ~ 10 ms, as predicted by the beat-frequency model for GX5-1, is in accordance with the evolutionary model^{42,43}. The magnetic dipole field strength of PSR1953+29 ($\approx 2 \times 10^{10}$ G; refs 45, 54) is comparable to that predicted by

Table 1 Best-fit parameters of power spectra

<i>N</i>	97	425	464	288	208	128
I_{lim} (c.p.s.)	2,277–2,486	2,486–2,695	2,695–2,904	2,904–3,113	3,113–3,322	3,322–3,531
$\bar{I}$ (c.p.s.)	2,427	2,603	2,790	3,001	3,225	3,403
ν_c (Hz)	20.07 ± 0.15	24.38 ± 0.16	27.26 ± 0.2	31.25 ± 0.5	35.11 ± 1.0	36.35 ± 1.6
λ (Hz)	4.2 ± 0.6	7.6 ± 0.5	9.2 ± 0.7	12.6 ± 2.0	12.4 ± 4.0	12.6 ± 10.0
P_{peak} (10^4 (c.p.s.) ²)	2.47 ± 0.13	2.89 ± 0.13	3.27 ± 0.2	3.33 ± 0.4	2.03 ± 0.6	2.02 ± 1.0
f_{peak} (%)	6.48 ± 0.17	6.53 ± 0.15	6.48 ± 0.2	6.08 ± 0.4	4.42 ± 0.7	4.18 ± 1.0
γ (s)	0.367 ± 0.04	0.247 ± 0.014	0.211 ± 0.014	0.216 ± 0.02	0.168 ± 0.04	0.188 ± 0.08
P_{LFN} (10^4 (c/s) ²)	2.32 ± 0.15	2.65 ± 0.10	2.83 ± 0.12	2.72 ± 0.19	1.93 ± 0.25	2.15 ± 0.4
f_{LFN} (%)	6.28 ± 0.2	6.25 ± 0.12	6.03 ± 0.13	5.50 ± 0.19	4.31 ± 0.3	4.31 ± 0.4
A	2.011 ± 0.009	2.004 ± 0.004	1.987 ± 0.004	1.983 ± 0.005	2.003 ± 0.007	2.002 ± 0.010
χ^2_ν (506 d.f.)	1.17	1.07	1.03	1.09	1.07	1.05

Results of least-squares fits of equation (1) to averaged power spectra taken from six source-intensity intervals. *N* is the number of individual power spectra (2-s data blocks) averaged, the values for I_{lim} indicate the limits in source intensity defining the intervals, and $\bar{I}$ is the average intensity within each interval. Peak centroid frequency ν_c , full-width at half-maximum λ , steepness parameter γ of the low-frequency noise and the white-noise level A are defined by equation (1). P_{peak} and P_{LFN} are the integrated excess power in the peak and in the low-frequency noise, respectively. These numbers are the integrals ($\nu=0$ to ∞) of the third and the second term, respectively, of the fits of equation (1), to the power spectra, normalized to assign a power a^2 to a sinusoidal modulation with amplitude a . f_{peak} and f_{LFN} are the corresponding relative amplitudes. Quoted errors correspond to the extreme limits of the $\chi^2_{\text{min}} + 1$ error contours.

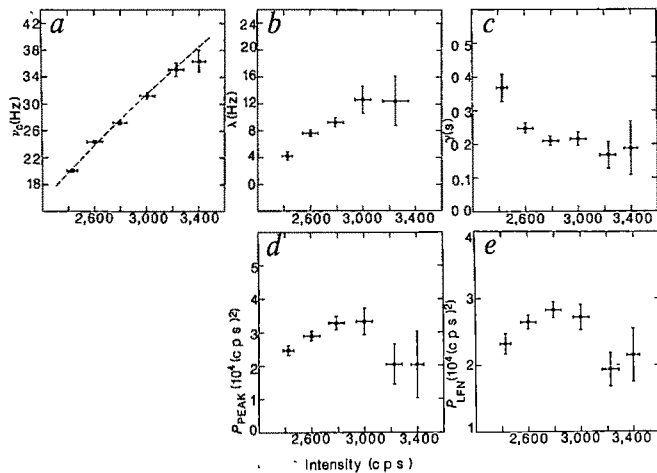


Fig. 5 Parameters of the power spectra (see Table 1) as a function of source intensity. *a*, Centroid frequency ν_c of the peak in the power spectrum. The dashed curve is a fit of equation (4) to the points. Clearly, a linear relation would also be an excellent fit to our results. *b*, Full-width at half maximum, λ , of the peak in the power spectrum. In this panel the two highest intensity intervals have been combined. *c*, Steepness parameter, γ , of the low-frequency noise. *d*, Integrated excess power of the peak in the power spectrum. Note the striking similarity with *e*. *e*, Integrated excess power of the low-frequency noise. Note the striking similarity with *d*. Vertical error bars are 1σ single-parameter, and take correlations between parameters in the fit into account. Horizontal bars represent the standard deviations of the intensity distributions in each of the six chosen intervals.

the beat-frequency model for the neutron star in GX5-1. These coincidences make the beat-frequency model attractive, but they could well be accidental.

Magnetic fields of neutron stars probably decay on a timescale of $(2-5) \times 10^6$ yr⁵⁵ (but see ref. 56). Therefore, if the quasi-periodic oscillations in GX5-1 are related to a magnetosphere (with a magnetic field strength of the neutron star of the order of 10^{10} G), the age of the neutron star in GX5-1 is probably of the order of 5×10^7 yr. Van den Heuvel and Taam⁵⁷ pointed out that, in the above evolutionary model^{42,43}, neutron stars are most probably formed during the mass-transfer phase by the accretion-induced collapse of a white dwarf. The mass-transfer phase in systems with an evolved companion lasts only $\sim 10^8$ yr. Thus, neutron stars in such systems could be relatively young ($< 10^8$ yr). The mass-transfer phase in systems with low-mass unevolved companions can last up to $\sim 10^9$ yr. Thus in those systems the probability of finding a relatively young neutron star is small. Therefore, if magnetospheric models are correct and the above evolutionary scenario is applicable, one would expect that similar quasi-periodic oscillations in low-mass X-ray binaries may be observed preferentially in systems which have an evolved companion star.

Our data suggest that the low-frequency noise is a different manifestation of the same phenomenon that causes the oscillations (notice the similarity between Fig. 5*d* and *e*). If we speculate that the X-ray oscillations are the result of 'blobs' in the inner accretion disk, the low-frequency noise could perhaps represent the size distribution of the blobs, larger blobs contributing to the noise power at lower frequencies. The blobs would have to disappear when the source intensity is high (decline of low-frequency noise). In this scenario, we can derive a lifetime for the blobs of 0.1–0.2 s from the observed width of the peaks in the power spectra. This estimate is based on the assumption that during its lifetime a blob causes a wavetrain of constant amplitude and frequency. We observe (see Fig. 5*b*, *c*) that when the steepness of the low-frequency noise is at a maximum (many large blobs present), the peak in the power spectrum is relatively narrow (long blob lifetime). This is contrary to what would be expected if this lifetime is dictated by differential keplerian rotation⁵⁸.

Possibly there is a 'gating' mechanism which allows incoming blobs (producing the low-frequency noise) to accrete in a piecemeal fashion (producing the quasi-periodic oscillations). This mechanism could then 'exhaust' the blobs, and now larger blobs may survive longer than smaller ones, consistent with our observations. A gating mechanism would probably fit in any of the above magnetospheric models^{20,21,38}.

Clearly, the scenarios and ideas discussed here are speculative. Any successful model must produce a physical mechanism explaining the observed properties of the quasi-periodic oscillations and the low-frequency noise, including their strong dependence on the source intensity.

We observed GX5-1 again for ~ 8 h on 29 April 1985. An analysis, performed the same day, showed that the quasi-periodic oscillations were again present. When this manuscript was in the final stage of typing, Dr W. Friedhorsky and his co-workers informed us that they observed (personal communication) similar phenomena from ScoX-1 as described here for GX5-1.

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Note added in proof: Models for the quasi-periodic oscillations along the lines suggested at the end of this article have been proposed by Alpar, Lamb and Shaham⁵⁹ and by Berman and Stollman⁶⁰. Intensity-dependent quasi-periodic oscillations have also been detected in CygX-2 (Hasinger *et al.*, IAU Circ. 4070), and quasi-oscillations have been demonstrated to be intensity dependent in ScoX-1 (M.v.d.K. *et al.*, IAU Circ. 4068). We point out that CygX-2 has an evolved companion, which supports our suggestion that the oscillations will be preferentially observed in such systems. We thank Fred Lamb for a useful discussion concerning the coherence time of the oscillations.

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How the geomagnetic field vector reverses polarity

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A highly detailed record of both the direction and intensity of the Earth's magnetic field as it reverses has been obtained from a Miocene volcanic sequence. The transitional field is low in intensity and is typically non-axisymmetric. Geomagnetic impulses corresponding to astonishingly high rates of change of the field sometimes occur, suggesting that liquid velocity within the Earth's core increases during geomagnetic reversals.

THE Steens Mountain (Oregon) reversed-to-normal polarity transition¹ is probably the most detailed record of a reversal of the geomagnetic field reported from a volcanic sequence. This Miocene reversal occurred 15.5 ± 0.3 Myr ago^{2,3} and can be correlated with the older boundary of marine magnetic anomaly 5 B2 (ref. 4). We have completed an extensive palaeomagnetic study of the Steens Mountain reversal to obtain a precise, detailed description of fluctuations in absolute palaeointensity of the geomagnetic field as it reverses. In the course of this study, we also obtained a more complete description of the directional changes during this reversal. These directional and palaeointensity data place new major constraints on the reversing Earth's dynamo.

Field and laboratory methods

We collected slightly over 1,000 oriented samples from three almost completely sampled sections, A, B and C, of the upper two-thirds of the Steens Basalt. Sections A and B are located only 1 km apart on Steens Mountain (42.63° N, 241.43° E) and are readily correlated by matching the transitional field directions. Taken together, these two sections span the end of the pre-transitional reversed period, the transition and the post-transitional normal period. Samples from section C (42.18° N, 240.07° E), located 130 km to the south-west of Steens Mountain, are reversed with the exception of those from the top flow, which display a transitional direction also observed on Steens Mountain¹ at the start of the reversal. The main eruptive centre was probably close to Steens Mountain, and the reversed part of Section C is believed to be older than the reversed zones sampled at the bottom of sections A and B, on the basis of the sequence of palaeomagnetic directions. The composite section obtained by combining sections A, B and C is 615 m thick and consists of ~120 distinct lava flows, which are numbered within each section from the top to the bottom.

The palaeodirection of the field was calculated from the remanence direction after either alternating field or thermal cleaning. After grouping successive flows with the same average direction of remanence, the Steens record consists of 55 directional groups (also numbered from the top to the bottom) of which 12 are reversed, 29 are transitional and 14 are normal (Fig. 1).

The absolute palaeointensity of the field was determined using the method of Thellier and Thellier⁵. Although time-consuming,

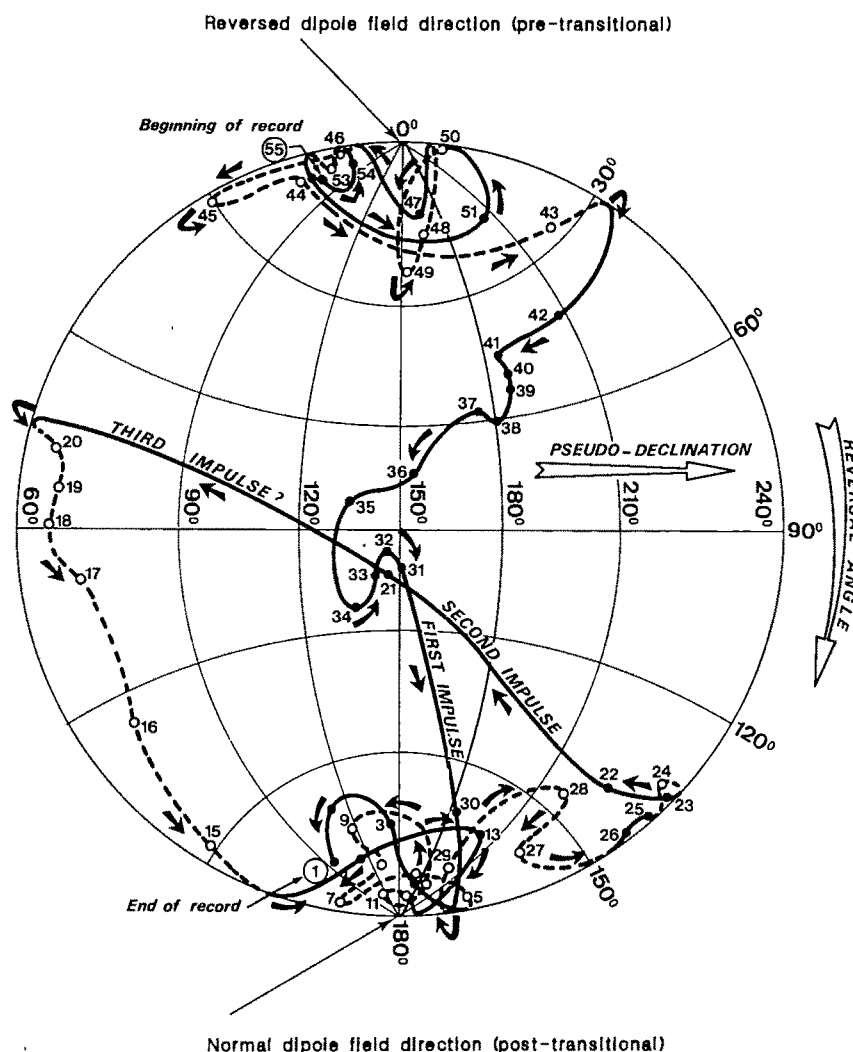
this method is preferable because it relies on experimentally well-established physical laws and a sound theoretical background. In addition, the method allows palaeointensities to be determined without the direct heating of samples to high temperatures required by other methods, which usually result in major magnetochemical changes. We selected 185 samples for palaeointensity investigations with low viscosity index, high Curie point and reversibility (or near-reversibility) of the strong-field magnetization curve versus temperature. These criteria proved to be pertinent. We obtained 157 useable palaeointensity values corresponding to 51 vectorial groups (group of successive flows with the same directions and palaeointensities).

Vectorial description

The transitional state of the geomagnetic field vector may be defined as corresponding to successive vectors of which either the directions or intensities (or both) are outside the range of secular variation observed in the adjacent pre- or post-transitional periods. Using this criterion, the end of the transition corresponds to directional group (DG) 15 whose direction and intensity are both transitional (Fig. 2). The beginning of the reversal was recorded by DG 43, which displays the first transitional palaeointensity (Fig. 2). However, the direction of the field at the same time was only 27° away from that of the reversed dipole (Fig. 1) and may be considered to be within the range of secular variation. Thus, transitional palaeointensities seem to have shortly preceded the transitional directions, which were recorded by the immediately following directional groups. In contrast, normal directions and intensities were recovered simultaneously at the end of the reversal.

Records of secular variation before and after the reversal were used to obtain a crude estimate of the duration of the transition. We assumed that these records are essentially complete because of the high extrusion rate needed to record the field reversal in such detail. A review of archaeomagnetic results⁶ shows that the angular rate of change of the virtual geomagnetic pole, when calculated from 2,000 yr BP to the present, is almost the same ($6 \pm 1^\circ$ per 100 yr) for areas as widely separated as Europe, North America and Japan. Assuming that this rate can be used for the Miocene, we found that the reversed and normal records in the Steens Basalt correspond to periods of 5,000 and 3,500 years, respectively. The corresponding accumulation rates of flows at Steens Mountain (sections A and B) are the same (43 ± 4 m per

Fig. 1 The Steens Mountain directional record. Stereographic projection (true angle) of the rotated field direction of the successive directional groups. Directions are rotated by 28.5° about the east-west horizontal axis to bring the dipole field directions coincident with the poles of the projection sphere³⁶. The reversal angle varies from 0° (reversed dipole direction) to 180° (normal dipole direction), and corresponds to lines of equal 'latitude' on the projection sphere. Lines of equal 'longitude' correspond to pseudo-declination, which is measured in the 'equatorial' plane from the projection of the north-seeking direction into this plane. A purely axisymmetrical field would have a pseudo-declination equal to 0 or 180° , which corresponds to near-sided or far-sided¹³ virtual geomagnetic pole paths, respectively. Directional group numbers are indicated near most of the circles representing the successive average field directions. With few exceptions (see Fig. 3), 95% confidence semi-angles are only a few degrees and could not be drawn on this diagram. Full circles and solid path are on the hemisphere of the projection sphere which contains the pole of projection (reversal angle 90° , pseudo-declination 150°); empty circles and broken path are on the opposite hemisphere.



1,000 yr) for the pre- and post-transitional zones, which makes it reasonable to estimate the duration of the reversal from the thickness of the transition zone (190 m). Hence, the transition period lasted $\sim 4,500$ yr, with an uncertainty of $\sim 1,000$ yr.

The pre-transitional reversed period is characterized by an average palaeointensity of $31.5 \pm 8.5 \mu\text{T}$ ($\pm$ s.d.). This mean is $\sim 30\%$ lower than the expected Miocene field at the site, as calculated from 49 independent palaeointensity data from all over the world, obtained using the Thellier method⁷⁻⁹. We interpret this difference, significant at the 95% confidence level, as resulting from a long-term decrease in the dipole intensity before the reversal. The rather large fluctuations of the field direction before the transition (Fig. 1) support the idea of a relative decrease of the dipole with respect to the non-dipole terms before the reversal. This pre-transitional dipole decrease would have lasted at least 5,000 yr.

In contrast, the intensity of the post-transitional normal field ($46.7 \pm 20.1 \mu\text{T}$) is not significantly different from the expected average Miocene field. However, large and apparently rapid intensity fluctuations are observed during this $\sim 3,500$ -yr-long record (Fig. 2), which may reflect some instability of the newly re-established dipole.

The major feature of the Steens Mountain transition is the succession of two distinct phases (Fig. 1). The first phase is a reversed-transitional-normal flip that apparently lasted no more than $\sim 1,500$ yr because of the thickness of the recording zone. The second phase is a normal-transitional-normal 'rebound'¹ which probably lasted twice as long. The apparently brief period of time between these two phases during which normal dipole-like directions occur is marked by an increase in field intensity back to pre-transitional values (Fig. 2). Thus, we interpret these

directions as corresponding to a briefly re-established dipole configuration.

During phases 1 and 2, we found very low values of geomagnetic intensity with several minima close to $5 \mu\text{T}$. The average intensity of the typically transitional field vectors, that is, the 18 vectors directed more than 45° away from the unsigned dipole direction, is $10.9 \pm 4.9 \mu\text{T}$. This is about one-fifth of the intensity of the non-transitional Miocene field. We observe also that the r.m.s. values of the north-south, east-west and vertical components of these transitional vectors are of a comparable order of magnitude with those of the historical non-dipole field at the latitude of the site. Figure 2 shows that there is a fairly regular westward movement of the field vector as the reversal develops, which strongly suggests that at least part of the transitional field is longitudinally drifting.

Transitional geomagnetic impulses

Three large directional jumps are observed during the transition (Fig. 1), amounting to $\sim 90^\circ$ differences between successive pairs of directional groups. This is in marked contrast to most of the Steens Mountain record, where successive angular differences are more often close to $\sim 20^\circ$, suggesting a detailed sampling of the transitional field. Although we first interpreted these jumps as a result of some intermission in the eruptive process, further observations suggested that they instead reflect some large and very rapid changes (geomagnetic impulses) of the field. The most convincing evidence comes from the second impulse, which is recorded in section A from flows A46-A43 (DG 24) to A40-A38 (DG 21). Two lava flows, A42 (DG 23) and then A41 (DG 22), erupted at the beginning of this impulse. After magnetic cleaning, the top and middle of both of these flows possess

distinctly different directions of magnetization (Fig. 3). Considering the chronology of the flows and their directions of remanence, these differences are not caused by reheating by the overlying flow. Field evidence also eliminates differences resulting from volcanological processes such as filling of lava tubes or rotation of magnetized blocks during eruption.

The directions of natural remanent magnetization can sometimes be extremely scattered, even antipodal, within a flow interior because of some secondary chemical remanent magnetization developing after the cooling of the flow¹⁰. In the only such example yet reported, thermal cleaning at high temperature allows the recovery of the direction of primary remanence¹⁰. In contrast, natural remanent magnetization directions in the interior of both flows A41 and A42 are not extremely scattered before or after magnetic cleaning (Fig. 3). The directions of magnetization of the near-surface samples do not change significantly during either alternating field or thermal cleaning. Most of the blocking temperature ranges of these samples are between 500° and 550 °C, whereas the interior samples display a distribution of blocking temperatures between 20 and 600 °C. However, after destroying some secondary magnetization directed towards the present-day field, directions found at high temperatures (~500 °C) in the interior samples are not different from those obtained by alternating field cleaning, and their cleaned directions remain obviously different from those of the near-surface samples (Fig. 3). Thus, the remanence of the interior samples seems to be primary in origin, having been acquired at high temperature during flow cooling.

Therefore, we interpret the distribution of directions observed in flows A42 and A41 as resulting from a geomagnetic impulse occurring during the cooling of these flows. The near-surface directions of flows A42 and A41 yield an average inclination of 45.4°, a declination of 279.5°, with $\alpha_{95} = 3.1^\circ$. This direction differs from the interior average directions of flows A42 and A41 by $16 \pm 11^\circ$ and $34 \pm 13^\circ$, respectively (95% confidence intervals). To estimate the rate of change of the field during the impulse, we need to know the cooling history of the flows. This is only possible for flow A41, which must have acquired its magnetization before being covered by the subsequent flow which has a different magnetization direction. Assuming that the primary remanence was acquired at ~500 °C, and according to temperature measurements in Hawaiian lava lakes¹¹, we find that ~8 months should have elapsed between the time that the top and the middle of this 4-m-thick flow became magnetized. The angular rate of change would have then been $\sim 50 \pm 20^\circ \text{ yr}^{-1}$. The 95% confidence interval given here corresponds only to the uncertainties about directions, neglecting those about the cooling time, which are large but difficult to estimate. Four palaeointensity values were obtained from flows A42 and A41. Their arithmetic average is $7.1 \pm 0.9 \mu\text{T}$, indicating that this impulse occurred when the field intensity was very low. The corresponding rate of change of the field vector during the impulse would have been $6.7 \pm 2.7 \mu\text{T yr}^{-1}$.

There is similar, though qualitative, evidence that the first directional jump (Fig. 2) also corresponds to a geomagnetic impulse. The possibility that the third jump also results from an impulse cannot yet be demonstrated.

General characteristics

Comparison of sedimentary records of the Brunhes–Matuyama reversal obtained from various locations on the globe indicates that the transitional field is not dipolar^{12,13}, insofar as the reversing field is faithfully recorded in the rocks studied. To describe the transitional fields in more detail, one has to turn to volcanic rocks, which are much more reliable recorders of both the direction and intensity of the field. Indeed, only volcanic rocks allow the determination of both the 'instantaneous' field direction and absolute palaeointensity. A consideration of the new data from the Steens Mountain reversal together with other data from transitions recorded in Cenozoic volcanic sequences confirms that transitional fields are low in intensity and suggests that these fields have three other major characteristics: import-

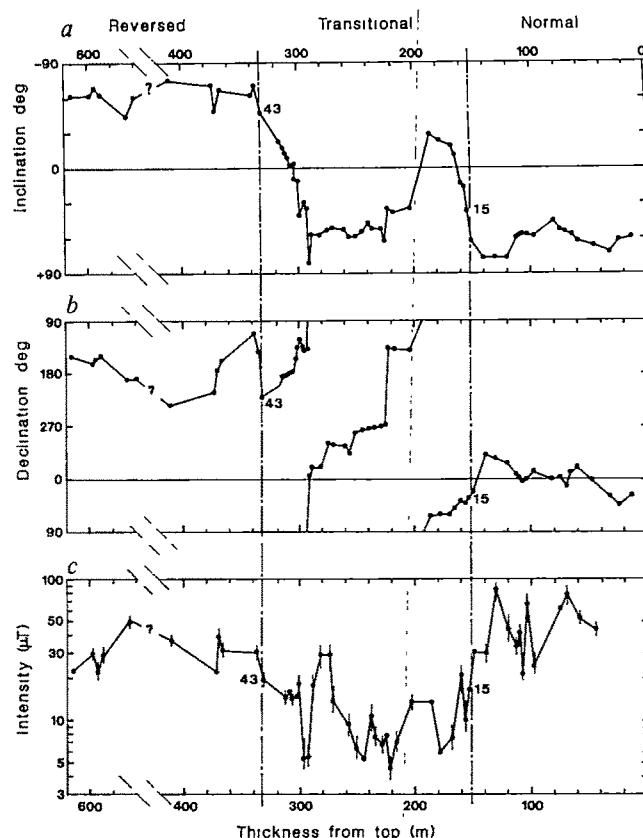


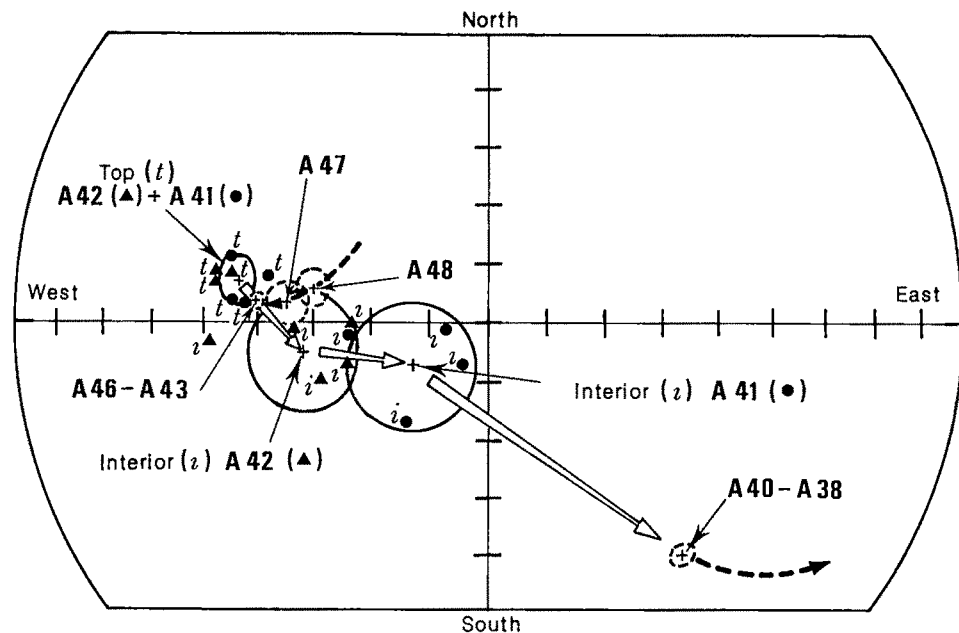
Fig. 2 The Steens Mountain vectorial record, showing average inclination, declination and intensity (logarithmic scale) of the geomagnetic field calculated for groups of successive lava flows which do not differ significantly in direction or intensity. *a*, *b*, 65 data points, some of which correspond to subdivisions of the directional groups within which intensity varies significantly between successive lava flows. *c*, 51 data points corresponding to the vectorial groups defined in the text. Group numbers 15 and 43 are indicated by the representative points. Average values are plotted versus thickness from the top of section A (2,950 m), so that time develops from the left to the right on the diagrams. No samples were collected between 410 and 560 m from the top. Error bars in *c* are s.e.m.; no bar indicates a single intensity determination. Uncertainties about inclination and declination are too small to be represented.

ance of non-zonal terms; occurrence of rapidly dumped oscillations; and geomagnetic impulses.

The Steens Mountain data confirm that palaeointensity of the geomagnetic field is low during reversals and suggest that a long-term dipole decrease precedes the transition. We did not find any evidence of the large intensity value thought to occur at the start of the first phase of the transition¹⁴. To what extent the average intensity of transitional fields found at Steens Mountain (~11 μT , or 20% of the non-transitional intensity) is typical is unknown because there are no comparable studies. Note that the average palaeointensity calculated from Cenozoic field reversals and excursions recorded in volcanic sequences from Iceland is only $5.5 \pm 3.1 \mu\text{T}$ (ref. 15), which suggests that average transitional palaeointensities are latitude dependent.

The importance of non-zonal components during the Steens Mountain reversal can be qualitatively understood from Fig. 1. Only sectorial or tesseral spherical harmonics can account for any deflection of the field direction from the north–south vertical plane (corresponding to pseudodeclination of 0 or 180° in Fig. 1). The second phase of the transition, which is also the longest, exhibits the directions that are the farthest from the geographical meridian. Considering the transition as a whole, the vector density of typically transitional directions (that is, $>45^\circ$ away from the unsigned dipole direction) is at a maximum for angular deviations between 30 and 40° from the geographical meridian (Fig. 4). Thus, most of the Steens Mountain transition is not

Fig. 3 Lambert equal-area projection of the cleaned directions of remanence of individual samples from lava flows A41 and A42, which erupted during the second geomagnetic impulse, and average directions of some pre- and post-impulse lava flows. Average directions are indicated by crosses with the corresponding α_{95} circles: solid circles, flows A41 and A42; broken circles, pre- and post-impulse lava flows. The pre-impulse directional path (broken line) is recorded successively by flows A48, A47, A46 to A43 and the tops of flows A41 and A42. Two successive records of the field direction during the impulse are provided by the interiors of flows A42 and A41. The post-impulse field direction is recorded by the overlying lava flows A40 to A38.



axisymmetrical, which is a general characteristic of Cenozoic reversals (Fig. 4). As well as the Steens Mountain reversal, data from the 71 directional groups in Fig. 4 correspond to transitions recorded by volcanic sequences in North America¹⁶⁻²⁰, Iceland^{21,22}, Japan²³ and Africa²⁴. Vector density is at a maximum for angular deviations between 40 and 70° from the geographical meridian, in marked contrast to the distribution of non-transitional field directions which would have typically displayed a maximum for a deviation of zero. The observed distribution indicates that, on average, east-west (non-zonal) components of transitional fields are at least as important as the components lying within the geographical meridian. As the latter components are a combination of zonal and non-zonal terms, this suggests that zonal and non-zonal components of transitional fields are of the same order of magnitude.

Thus, during polarity transitions, the geomagnetic field not only loses its dipolar morphology but also its axisymmetry. Similarly, the present-day non-dipole field does not show any predominance of zonal terms. The absence of a purely zonal morphology for transitional fields is compatible with the sugges-

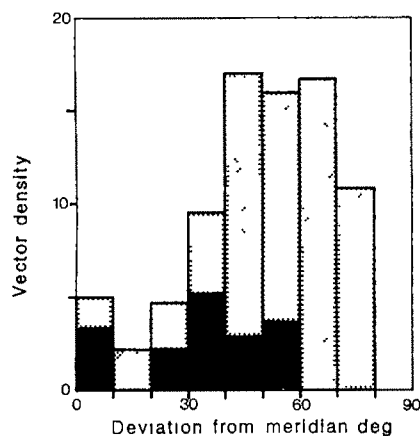


Fig. 4 Histogram showing vector density versus deviation from the geographical meridian for 71 transitional directions from Cenozoic reversals recorded by volcanic sequences (see text). All transitional directions considered are flow or directional group averages which are more than 45° away from the dipole field direction at the sampling site. Vector density is calculated using one as the value of the area on the sphere corresponding to the 40-50° range in deviation from the meridian. Black columns, data from the Steens Mountain transition.

tion that the frozen-flux approximation is valid during reversals²⁵. However, this agreement does not imply that flux diffusion is negligible over the full time of a reversal. In particular, the beginning of the Steens Mountain reversal, which seems to be only moderately non-zonal (Fig. 1), could result from ohmic decay within a geodynamo temporarily inoperative as a consequence of the axial symmetry²⁶.

The notion that the transitional state of the geodynamo corresponds to an oscillating field was proposed by Braginsky²⁷. In contrast, more recent models suppose that the field reverses in a progressive way, through either some spreading of the reversal over the core surface^{13,28} or by reversible energy transfer from the dipole to non-dipole terms²⁹. The entire directional path observed during the Steens Mountain transition can be interpreted as corresponding to a rapidly damped oscillation of the field. Although other records of reversals by volcanic rocks are not as detailed as the Steens Mountain record, examples have been reported in which the field reverses polarity and then comes back to its initial polarity before reversing again, even in such incomplete records^{19,30}. Such observations favour models of the geodynamo that allow a highly damped oscillatory state of the geomagnetic field during reversals.

The geomagnetic impulses occurring during the Steens Mountain transition can be considered as impulses of the first order time-derivative of the field. They may be interpreted as large and possibly global changes of the field configuration with time constants of the order of 1-2 yr. By comparison, we note that the same order of magnitude has been proposed recently for the smoothing time of the 1969 impulse resulting from filtering by the mantle³¹. The rates of change of the field components during the Steens Mountain transitional impulses would have been very large. For the second impulse, the rates of change of the three components would have been between 15 and 50 times larger than the maximum rates calculated for the historical non-dipole field³².

Obviously diffusion of field lines cannot explain such rapid changes; even for harmonic degrees as large as 10, the decay time is still equal to a few hundred years³³. Larger-degree harmonics with decay times of the order of a few years require implausibly large fields at the core-mantle boundary. Under the frozen-flux approximation, however, the transitional geomagnetic impulses can be accounted for by a large increase in fluid velocity at the core surface. This suggests an increase in the level of turbulence within the liquid core during reversals. This increase in kinetic energy can be of thermal origin, resulting from too steep a thermal gradient within the core for a convective

regime to be preserved³⁴. Alternatively, this increase can result from a decrease in magnetic energy. We have shown that, on average, the intensity of the geomagnetic field at the Earth's surface reduces to one-fifth of its normal value during the Steens

Mountain polarity transition. If the field intensity within the core also decreases during reversals, the liquid can become more unstable as part of the magnetic energy is transferred into kinetic energy³⁵.

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Primary structure and transmembrane orientation of the murine anion exchange protein

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The amino-acid sequence of murine band 3, deduced from the nucleotide sequence of a complementary DNA clone, confirms that this integral membrane glycoprotein is composed of two major structural domains which correlate with its dual functions as the anchor for the erythrocyte cytoskeleton and as a plasma membrane anion antiporter. This latter activity resides within a highly hydrophobic domain that crosses the plasma membrane at least 12 times.

BAND 3 is the major integral membrane glycoprotein of the mammalian erythrocyte¹. It functions as an anion antiporter, mediating the one-for-one, obligatory and electroneutral exchange of chloride and bicarbonate across the plasma membrane (see refs 2-6 for review). This rapid process ($t_{1/2}$ = 40-60 ms), catalysed by the ~1,000,000 molecules of band 3 per erythrocyte³, increases the capacity of the blood to transport CO₂ from the respiring tissues to the lungs. Through its tight association with ankyrin^{7,8}, band 3 anchors the mesh-like cytoskeletal network of spectrin and ankyrin to the inner surface of the erythrocyte plasma membrane (see ref. 9 for review).

As a consequence of its physiological importance and relative abundance, band 3 structure and membrane topology have been characterized extensively by biochemical techniques including proteolytic mapping, inhibitor binding and covalent modification (see ref. 4 for review). Band 3, 929 amino acids long, has two distinct structural domains, one containing the anion exchange activity and the other involved in cytoskeletal interactions.

The ~400 N-terminal amino acids of band 3 are hydrophilic and face the cytoplasm. This negatively charged domain possesses the high-affinity binding sites for ankyrin⁸ as well as, in human band 3, haemoglobin¹⁰ and several glycolytic enzymes^{11,12}. Removal of the cytoplasmic domain of band 3 by proteolytic cleavage of erythrocyte ghosts results in no loss of anion transport activity¹³, demonstrating that this function resides entirely within the C-terminal ~500 amino acids of the protein. Studies on the topology of this domain suggest that it traverses the membrane at least seven times^{14,15}; hitherto it has

only been possible to obtain the sequence of small fragments of this region in the human protein.

Here we report the isolation and the sequence of cDNA clones encoding the full length of murine band 3 messenger RNA. This is the first plasma-membrane anion transporter to be sequenced. Previous studies have established that human and murine band 3 have very similar sizes and biochemical properties¹⁶. We propose a model for band 3 topology that incorporates data from many biochemical and physiological studies.

Isolation of cDNA clones

In mice, anaemic stress induces proliferation in the spleen of erythroid precursor cells that actively synthesize erythrocyte proteins^{17,18}. Polyadenylated RNA from these spleens was used to construct a cDNA library in the expression vector λ gt11 (ref. 19). We screened this library with a polyclonal antibody²⁰ against purified mouse erythrocyte band 3, initially revealing several positive clones, the inserts from which hybridized to each other and to a 4,300-nucleotide mRNA in total spleen RNA (data not shown). The longest of these, pB33, contains a 1,800-base-pair (bp) insert, which was shown subsequently to encompass only ~280 bp of the C-terminal protein coding region, but which extends throughout the entire 3' untranslated region (1,500 bp). We used pB33 as a hybridization probe to screen a second cDNA library, constructed to optimize the yield of full-length transcripts (see Fig. 1 legend). Sequencing the longest insert (clone pB399; 2.7 kilobases (kb)), revealed that it did not contain the N-terminus of native band 3, judged by the homology of the deduced amino-acid sequence with that of the N-terminus

[illegible]

Fig. 1 Composite nucleotide sequence of the cDNA corresponding to mouse band 3 mRNA showing the deduced amino-acid sequence. Nucleotide numbers are indicated in the right-hand margin and the sequence is written with the 5' end of the cDNA at the top left. Position 1 is the ATG triplet which is the probable start of the polypeptide. The in-frame nonsense codon at base -63 and an out-of-frame ATG at -50 are underlined. The two potential glycosylation sites at (611 and 660) are designated by boxes. The 15 tandem repeats of the tetranucleotide ATTC at nucleotide positions 3,490-3,550 in the non-translated region and the polyadenylation signal at base 4,232 are underlined.

Methods. Molecular cloning of mouse band 3. Total poly(A)⁺ RNA was isolated from fresh spleens of severely anaemic BALB/c mice by homogenization in 5 M guanidinium isothiocyanate, purification by ultracentrifugation through a CsCl gradient³⁷ and affinity chromatography on oligo(dT) cellulose³⁸ of this RNA, 10 µg was used to construct a cDNA library in the bacteriophage expression vector λgt11¹⁹ as described previously³⁸, except that the endogenous *EcoRI* sites were not protected with *EcoRI* methylase. A polyclonal rabbit antibody against mouse erythrocyte band 3 (ref. 16) was used in conjunction with ¹²⁵I-labelled protein A to screen⁴⁰ 10⁶ recombinant phage plaques. Fifteen antibody-positive clones were identified and subcloned into the plasmid vector, pUC13⁴¹. pB33 contains an 1,800-bp insert, the longest clone isolated from this library. The *EcoRI* fragment containing the entire pB33 insert was purified by agarose gel electrophoresis and labelled with [α-³²P]dCTP by nick-translation⁴². This was used to probe a second mouse spleen λgt11 cDNA library, which was made as follows: 10 µg poly(A)⁺ RNA was used to synthesize double-stranded cDNA essentially as described by Gubler et al.⁴³ This cDNA was treated with *EcoRI* methylase³⁹ and size-selected by agarose gel electrophoresis to exclude fragments <2.5 kb in length. Of the recombinant phage in this library, 1% contained inserts 3.5 to 4.3 kb long which hybridized to the pB33 insert. pB399 is a pUC13 subclone of the longest isolate obtained from this library. pB3SP4 is a pSP65 subclone⁴⁴ of the longest isolate obtained by screening the library with a 500-bp fragment *EcoRI*/PvuII from the 5' end of pB399. DNA sequencing. Three 200-bp fragments of pB3SP4 were sequenced by chemical cleavage⁴⁵ of DNA fragments end-labelled with [α-³²P]-nucleotides using the Klenow fragment of DNA polymerase⁴². All other sequences were obtained by subcloning cDNA fragments into M13 and sequencing by the dideoxy chain-termination method⁴⁶ using [α-³⁵S]dATP⁴⁷. These fragments were obtained by progressive *Bal31* deletion⁴⁸ in the case of pB33, or by random fragments generated by sonication for pB399 and pB3SP4. *Bal31* digestions were performed as follows: 25 µg plasmid was digested to completion with *NarI*, which cuts pB33 at a unique site 175 bp from the 3' end of the band 3 insert. The linearized DNA was digested with *Bal31* (New England-Biolabs) for 24 min at 30 °C using a concentration of 1 U *Bal31* per µg DNA. Aliquots of the reaction mixture were removed at 3-min intervals. Following ethanol precipitation, the DNA was digested with *EcoRI* and ligated with M13 mp8⁵⁰ RF DNA digested previously with *EcoRI* and *SmaI*. Preparation of cDNA fragments for sonication was as follows: 5 µg gel-purified restriction fragment was self-ligated and sonicated⁴⁹; the ends were repaired by treatment of the fragments with T₄ polymerase in the presence of 0.1 mM deoxynucleoside triphosphates. The resulting DNA was fractionated on a 1.5% agarose gel. The eluted fragments were ligated with *SmaI*-cut M13 mp18 RF DNA. All transformations were done with *Escherichia coli* JM103 (ref. 50) as described previously⁴². The entire sequence was assembled using the DB programs of Staden⁵¹. The database contains 174 gel readings, representing an average nucleotide redundancy of 5. The entire sequence has been obtained for both strands.

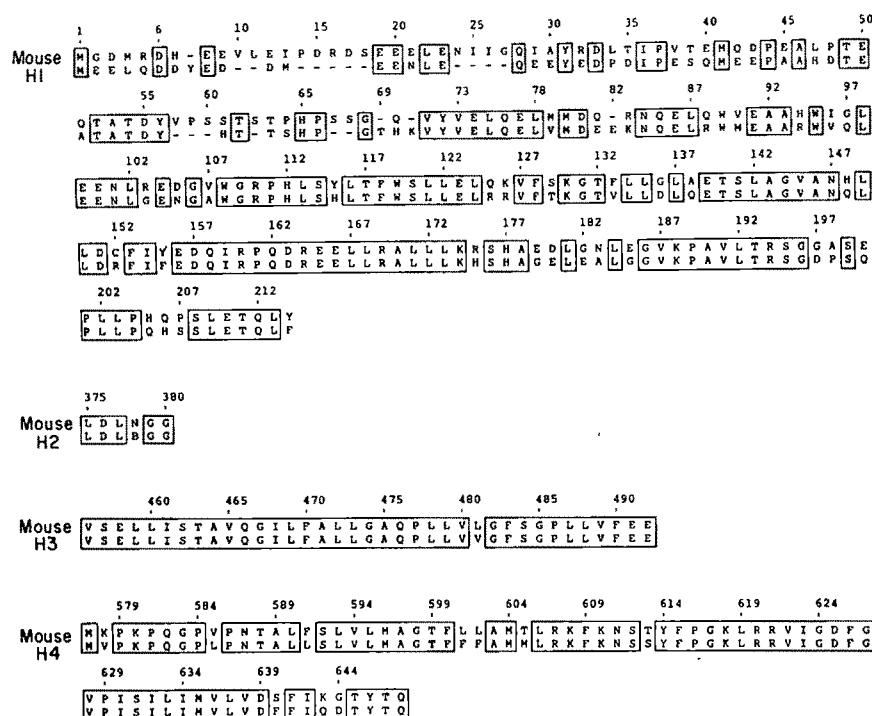


Fig. 2 Alignment of sequences of fragments of human band 3 with the deduced sequence of the mouse protein. The ALIGN program of Dayhoff *et al.*⁵² was used to obtain the optimal alignment using the mutation data matrix⁵². Shown are the alignments for an N-terminal fragment, H1 (ref. 21), a short peptide from the N-terminus of a M_r 14,000 chymotryptic fragment, H2 (ref. 25), and two fragments from the membrane-associated domain, H3 (ref. 25) and H4 (ref. 26). Boxes indicate amino acid identity between the two species. The alignment scores were compared with those obtained from 150 randomizations of the entire mouse sequence, using a break penalty of 2. The scores for the alignments were 14.6 (H1), 2.66 (H2), 15.5 (H3) and 12.2 (H4) standard deviations, respectively.

of human erythrocyte band 3 (ref. 21; see below). Therefore, we re-screened the library using a 5' fragment of pB399 as a probe, and isolated a cDNA clone, pB3SP4, encompassing 127 bp of 5' untranslated region as well as the entire 2,900-bp open reading frame encoding murine band 3.

Band 3 sequence homology

The complete sequence of 4,383 nucleotides compiled from the cDNA clones pB33, pB399 and pB3SP4 is shown in Fig. 1. The size of the full-length cDNA represented by the three clones agrees well with that of the mRNA (data not shown). The overlapping regions of the three clones have identical sequences. A single open reading frame extends for 929 codons from the ATG at nucleotide 1 to the TGA at base 2,919. There are two in-frame ATG codons at the beginning of the open reading frame (residues 1 and 4). We have assigned the first Met codon as the initiator because it is the first in-frame ATG downstream of the (in phase) stop codon at base -63. The sequences flanking this ATG, but not the other one, are homologous to the highly conserved sequence that flanks functional initiation sites in eukaryotic mRNAs: AXX AUG G²². The relative molecular mass (M_r) of the predicted polypeptide is 103,000, in good agreement with estimates of 90,000–100,000 obtained from SDS-polyacrylamide electrophoresis of the native human¹ and murine²³ proteins. The predicted C-terminal amino acid of the murine sequence is valine, which is also the C-terminal residue of the human protein²⁴. Indeed, of the 12 C-terminal residues predicted from the mouse cDNA sequence, 11 are identical with those determined by carboxypeptidase-Y digestion of human band 3 (R. Reithmeier, personal communication). Parts of the the amino-acid sequence deduced from the cDNA sequence contain high homology to that of the several known fragments of human erythrocyte band 3 (Fig. 2), proving that the clones we have isolated encode the murine erythrocyte anion exchange protein.

The most striking homology is between residues 455 and 492 of murine band 3 and the 38-amino-acid fragment from the human protein designated H3 (ref. 25) in Fig. 2. There is only a single conservative (Leu → Val) substitution between the two sequences; there are no insertions or deletions. Excellent alignment is also observed between the deduced sequence of murine band 3 and the sequence of a peptic fragment (P5) of the human protein designated H4 (ref. 26; Fig. 2); only 10 of the 72 residues differ and alignment of the sequences required no insertions or

deletions. The sequence of the N-terminal 201 residues of human band 3 (ref. 21), designated H1, exhibits a slightly lower homology with the sequence deduced from the murine band 3 clone.

Structural domains of band 3

A hydropathy plot²⁷ (Fig. 3) reveals that murine band 3 can be roughly divided into three domains. The N-terminal 420 residues constitute the hydrophilic cytoplasmic domain with a negative net charge (65 acidic and 37 basic residues). The central 450 amino acids are predominantly amphipathic, and are intimately associated with plasma membrane lipid. In this domain, groups of hydrophobic residues, which correspond to the peaks *a–j* in Fig. 3, are interspersed with polar, predominantly basic, residues, depicted schematically in Fig. 4. The extreme 32 C-terminal residues of band 3 may constitute a third domain. Eleven of these are either Glu or Asp, making it unlikely that the C-terminus is buried within the lipid bilayer.

Cytoplasmic domain

The binding of haemoglobin¹⁰ and glycolytic enzymes^{11,12} to the cytoplasmic domain of human band 3 is mediated by the first 11 residues of the band 3 polypeptide²⁸. The apparent absence of glyceraldehyde 3-phosphate dehydrogenase from mouse erythrocyte ghost preparations (V. Patel, personal communication) is consistent with the lack of homology between the mouse and human sequences in this N-terminal undecapeptide.

Other features of the cytoplasmic domain are more conserved: both human²⁹ and murine band 3 possess six Cys residues (Fig. 4). Three of the murine band 3 cysteines are in the cytoplasmic domain (CH65), consistent with studies on human band 3 (ref. 29).

Low *et al.*³⁰ propose that the cytoplasmic domain of CH65 is a homodimer with a regulated hinge rich in proline residues. These prolines apparently disrupt the α -helical structure of the polypeptide, rendering it susceptible to proteolysis. Note that these prolines (at positions 161, 188, 201 and 204) are all conserved between human and murine band 3 (compare with Fig. 2); Fig. 4 shows them to be clustered around a trypsin cleavage site at position 194. (Interestingly, two other proline-rich regions in the predicted mouse sequence are found at the known primary sites of intracellular and extracellular proteolysis, between residues 361–368 and 567–586, respectively.)

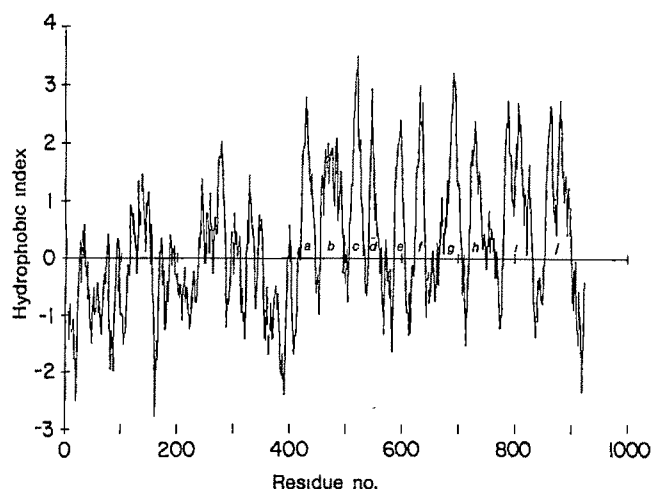


Fig. 3 Hydropathy plot of the deduced amino-acid sequence of murine band 3 using the algorithm and hydropathy values of Kyte and Doolittle²⁷. Hydrophobicities were averaged over windows of 11 amino acids. The 10 hydrophobic regions are designated a-j.

Between the proposed hinge and a region rich in tryptophan (residues 89–119), also highly conserved, is the purported antigenic region of band 3 (ref. 30). Monospecific polyclonal antibodies against total human band 3 recognize determinants located within a M_r 20,000 fragment of the cytoplasmic domain³¹; this site is sufficiently distant from the N-terminus so that the binding of antibody does not interfere with the binding of haemoglobin³⁰. The region between residues 125–138 contains only 57% homology between human and mouse, whereas the residues flanking it on either side are 90–95% homologous, consistent with the lack of cross-reactivity of anti-human band 3 antisera with the mouse protein and vice-versa²³.

The ankyrin binding site on band 3 has not been identified; Low *et al.* tentatively place it between the hinge region and the immunoglobulin G region, because antibodies against band 3 compete with labelled ankyrin for binding³⁰. The high-affinity ankyrin-binding site is probably conserved among different

species and, perhaps, among different non-erythroid ankyrin-binding proteins.

Membrane-associated domain

The C-terminal half of band 3 contains 10 long hydrophobic stretches (Fig. 3a–j). Regions with hydropathy averages >1.5 are usually tightly associated with lipid, and, in the case of several proteins, span the membrane in an α -helical configuration^{27,32}. Our two-dimensional model indicates that seven of these peaks correspond to sequences of amino acids that span the phospholipid bilayer once (Fig. 3a, c–h) and that two (b, i), and possibly a third (j), span twice.

Peaks a, c, e, g and h, designated single-spanning regions 1, 4, 6, 8 and 9, respectively, correspond to continuous stretches of 20–24 extremely hydrophobic amino-acid residues and fit the criteria for forming membrane-traversing α -helices^{27,32}.

Chymotryptic digestion of intact erythrocytes cleaves band 3 into a carboxy-terminal glycosylated peptide, CH35, and an amino-terminal non-glycosylated CH65 (Fig. 4). Sequence data on the N-terminus of human CH35 (ref. 26) positions this site between the putative membrane-spanning segments 5 and 6, thus this segment must be extracytoplasmic. The extensive homology between the amino-acid sequences of murine and human band 3 in the peptic fragment studied by Brock *et al.*²⁶ allows us to assign the topological asymmetry to the corresponding membrane-spanning segments 5–8 of the mouse sequence. Of the two potential sites (Asn-X-Ser/Thr) for attachment of the single N-linked oligosaccharide, the one at residue 611 in the murine sequence corresponds to the site in the human protein²⁶ shown not to contain carbohydrate. Therefore, the site at Asn 660 must be the extracellular attachment site, consistent with the finding that the human Tyr 646 is a substrate for extracellular radioiodination²⁶.

The region encompassing segments 1–5 corresponds to part of the M_r 17,000–22,000 fragment, CH17, generated by chymotryptic digestion of ghosts. It must span the membrane an odd number of times as it results from both an intra- and an extracellular proteolytic cleavage³³. Studies with surface-specific radiolabelling reagents demonstrate that it traverses the bilayer at least three times¹⁵.

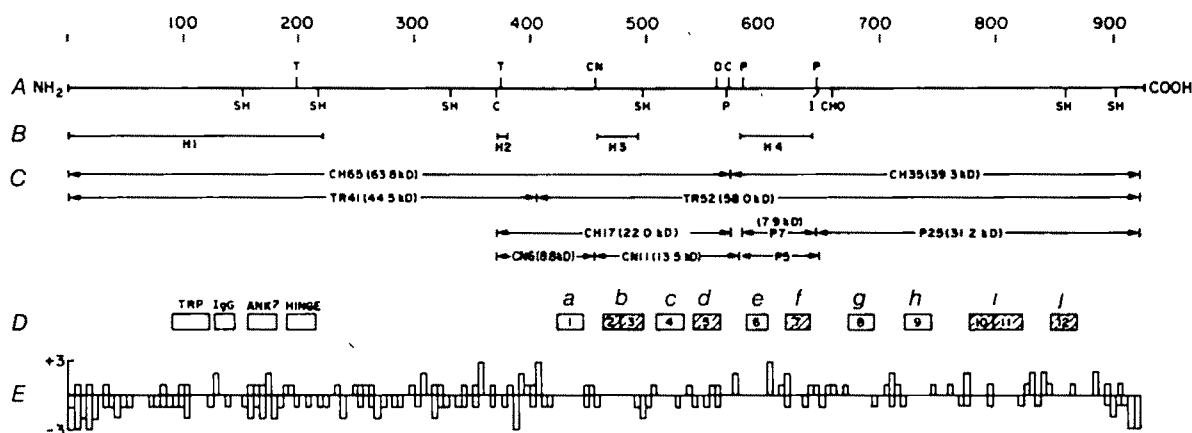


Fig. 4 Domain map of band 3. **A**, The scale is the complete amino-acid sequence of murine band 3 deduced from the nucleotide sequence. Probable sites for proteolytic cleavage by trypsin (T) at position 194 (R. Reithmeier, personal communication) and 381 (refs 15, 25), chymotrypsin (C)³³, cyanogen bromide (CN)^{15,25} and papain (P)¹⁴ are indicated. Cysteine residues (SH), binding site for H_2DID s (D), and the known site for exofacial radioiodination (I)²⁶ are all designated. The proposed site for attachment of the N-linked oligosaccharide (CHO) is shown. **B**, Alignment of the fragments from human band 3. The codes H1–H4 are defined in Fig. 2 legend. **C**, Primary fragments arising from digestion of human band 3 with extracellular chymotrypsin, CH65 and CH35^{13,24}; intracellular trypsin, TR41 and TR52²³; extracellular papain, P7 and P25¹⁴; extracellular pepsin, P5^{26,53}; and cyanogen bromide cleavage of CH17, CN6 and CN11^{15,25}. Numbers in parentheses are the actual M_r values of the fragments calculated from their amino-acid composition. These values do not take into account the contribution of the single N-linked oligosaccharide. **D**, Structural features within the N-terminal cytoplasmic domain are: tryptophan-rich (TRP)³⁰, antibody-binding (IgG)^{30,31}, ankyrin-binding (ANK) and hinge are indicated (see text). Locations of the hydrophobic peaks from the hydropathy plot (Fig. 3) are indicated by the letters a–j and the corresponding presumed membrane-spanning regions are designated by the numbers 1–12. Hatched boxes indicate that the region is likely to be amphipathic (see text). **E**, Charge plot. Location of charged residues in the mouse band 3 sequence is indicated by the bar graph, the positions corresponding to the sequence numbers at the top of the figure. The basic residues (Arg and Lys) are assigned a value of +1 while the acidic residues (Glu and Asp) are assigned a value of –1. The bars represent the total (+) or (–) values, above and below the horizontal axis, respectively, over 5 residues.

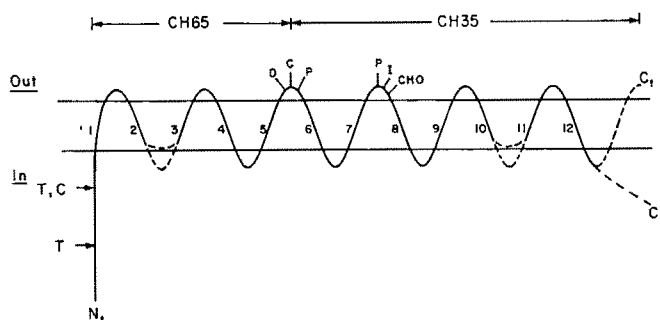


Fig. 5 Model for band 3 orientation in the plasma membrane. A two-dimensional projection of the orientation of the cytoplasmic domain and the membrane-spanning regions in the lipid bilayer is shown. The sites of oligosaccharide attachment (CHO), H_2DIDS binding, as well as the major extracellular sites of proteolysis, are indicated. Abbreviations are as described in Fig. 4 legend.

If we assume that peaks *a*, *c* and *d* of the hydrophobicity plot correspond to three membrane-spanning segments, 1, 4 and 5, then either region *b* (residues 453–491) does not span the membrane, or it must do so twice. Hydrophobicity peak *b* is quite broad, encompassing 38 moderately apolar residues. These residues can be arranged to form a structure composed of two antiparallel amphipathic helices with Pro 477 forming a turn within the membrane or just at the interface between the bilayer and the cytoplasm. Analysis of the helical amphiphilicity³⁴ suggests that both helices formed by this region of the protein would have all their polar residues projecting from a single face of each helix. Thus, we conclude tentatively that CH17 possesses five membrane-spanning α -helices, of which three (2, 3 and 5) are amphipathic. Helices 1 and 2 could form a hairpin that functions as the internal uncleaved signal sequence²⁰ for insertion of the nascent chain into the endoplasmic reticulum membrane.

Labelling of intact human erythrocytes with a reagent to which the membrane is impermeable has led to the identification of three exofacial lysines in CH17: one on the cyanogen bromide fragment CN6 and two on CN11 (see Fig. 4 and ref. 15). One of the lysines in CN11 binds extracellular 4,4'-diisothiocyanato-2,2'-dihydrostilbene disulphonate (H_2DIDS), a potent inhibitor of anion transport³⁵. Our topological model (Fig. 5) is consistent with these labelling studies, predicting that all three lysines in mouse CH17 (positions 449, 558 and 561) are exofacial. These

data also allow us to assign the site for H_2DIDS binding to either Lys 558 or Lys 561.

Virtually nothing is known about the transmembrane orientation of the C-terminus of band 3 corresponding to hydrophobic peaks *g*–*j*. Our model proposes four or five membrane crossings. The region corresponding to the 43 residues of peak *i* in Fig. 3 resembles membrane spans 2 and 3 in its ability to form an α -helical hairpin in which Arg 800 is situated just at the cytoplasmic surface and the C-terminal 24 amino acids return to the extracellular surface. The proline residues at positions 796 and 802 may be involved in forming the hairpin structure. These two spanning segments, along with segment 12, could form moderately amphipathic helices. Following an extremely polar stretch between residues 832 and 850, there is a short region of 18 mixed hydrophobic and polar residues that forms the N-terminal part of peak *j* in Fig. 3. This is the longest stretch of apolar amino acids between position 850 and the C-terminus of the protein. We propose that this segment spans the membrane once, as an amphipathic α -helix (Fig. 5, segment 12), positioning the C-terminus of the protein inside the cell. Alternatively, the protein may traverse the membrane once more, leaving the C-terminus outside the cell. These two possibilities are shown as dotted lines in the model (Fig. 5).

Analysis of the predicted sequence of murine band 3 reveals that the protein possesses 12 membrane-spanning regions. Five of these are hydrophobic and probably traverse the bilayer as single-spanning α -helices. The seven remaining membrane-spanning regions contain both hydrophobic and polar residues that cross the plasma membrane as amphipathic helical structures, confining their polar and charged side chains to a single face of the helix. These helices, oriented with their axes transverse to the plane of the membrane, could cluster, forming at least one hydrophilic region within the bilayer across which the charged residues involved in the anion-exchange process may pair. Clustering of amphipathic helices round an aqueous 'pore' has been proposed for other transport proteins which span the membrane several times^{32,36}. Significantly, either of the two lysine residues (558 or 561) which bind to stilbene disulphonate inhibitors of anion exchange are associated with such an amphipathic region.

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Is GX5-1 a millisecond pulsar?

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The bright galactic bulge source GX5-1 (4U1758-250) has recently been observed¹ to exhibit quasi-periods between 25 and 50 ms, with a correlation between the corresponding frequencies f and the count rate I given by $d \log f / d \log I \sim 2.5$. This is the first source observed to exhibit such a phenomenon. We propose here that this behaviour results from fluctuations in accretion rate onto a millisecond pulsar, which has been spun up by accretion to its steady-state spin rate. According to models that use spin-up by accretion to bring the neutron stars to steady-state fast short periods, galactic bulge sources are likely progenitors of millisecond pulsars²⁻¹⁰. After steady state is achieved in these systems, accretion may continue without further spin-up, so that for a substantial fraction of the lifetime of a galactic bulge X-ray source, the neutron star may already be spinning rapidly at the equilibrium spin. The behaviour of GX5-1 can be explained in terms of deviations from this equilibrium state resulting from changes in the accretion rate $\dot{M}$; GX5-1 may, therefore, be a new millisecond pulsar.

For a neutron star of mass M with a dipole magnetic field B_8 (in 10^8 G), radius R_6 (in 10^6 cm) and mass accretion rate $\dot{M}_{17}$ (in 10^{17} g s⁻¹), the Alfvén radius r_A (inner edge of the keplerian accretion disk) is given by

$$r_A = 1.1 \times 10^6 B_8^{4/7} R_6^{12/7} \dot{M}_{17}^{-2/7} (M/M_\odot)^{-1/7} \text{ cm} \quad (1)$$

We have used the normalization of ref. 2 in this equation. The keplerian angular velocity at r_A is

$$\Omega_K(r_A) = (GM)^{1/2} r_A^{-3/2} \\ = 9.5 \times 10^3 B_8^{-6/7} R_6^{-18/7} \dot{M}_{17}^{3/7} (M/M_\odot)^{5/7} \text{ rad s}^{-1} \quad (2)$$

where G is the gravitational constant. Let us denote the equilibrium values of $\dot{M}$ and r_A by $\dot{M}_0$ and r_A^0 . These values determine the equilibrium rotation rate of the star. If $\dot{M}$ deviates slowly from $\dot{M}_0$, the star accretes from the corresponding r_A of equation (1). The specific angular momentum of the accreted matter also varies with this 'instantaneous' r_A ; however, the star's rotation rate will not be affected by such torque variations on time scales much shorter than the spin-up time scale, $\approx 10^7$ yr. Our basic claim is that if the accretion process is controlled by the interaction (matching) between the magnetosphere co-rotating with the star and matter at the instantaneous Alfvén radius, then the luminosity will display a 'beat' frequency¹¹

$$\omega = \Omega_K(r_A) - \Omega_0 \quad (3)$$

in the observer's frame, where Ω_0 is the stellar steady-state angular frequency. The nature of this matching is discussed below.

If the observed X-ray flux originates entirely at the surface of the neutron star, its time dependence is simply that of $\dot{M}$, through $L = G\dot{M}\dot{M}/R$. The luminosity L is related to the observed count rate I by

$$L = 8.1 \times 10^{37} \text{ erg s}^{-1} \left(\frac{d}{10 \text{ kpc}} \right)^2 I(1,000 \text{ c.p.s.}) \quad (4)$$

where d is the distance to GX 5-1. Using equations (2), (3) and (4), we get

$$\omega = a I^{3/7} - \Omega_0 \equiv a(I^{3/7} - I_0^{3/7}) \quad (5)$$

Table 1 Estimated parameters for the neutron star

P_{obs} (ms)	I (1,000 c.p.s.)	r_A (10^6 cm)	$P_K(r_A)$ (ms)	$\Omega_K(r_A)$ (rad s ⁻¹)
25	3.1	4.5	5.20	1,200
50	2.4	4.84	5.84	1,080
	B (10^9 G)	r_A^0 (10^6 cm)	P_0 (ms)	Ω_0 (rad s ⁻¹)
	5.5	5.26	6.6	950

Alfvén radii and keplerian rotation rates corresponding to the observed quasi-periods 25 ms and 50 ms and the rotation period P_0 and magnetic field B are given. These values are obtained from equation (5), with $M = 1 M_\odot$ and $\dot{M}_{17} = 1$

where I is the count rate in 1,000 c.p.s. and a is given by $a = 2.3 \times 10^4 B_8^{-6/7} R_6^{-13/7} (M/M_\odot)^{2/7} (d/10 \text{ kpc})^{6/7}$. From the observed values of $P = 50$ ms at $I = 2,400$ c.p.s. and $P = 25$ ms at $I = 3,100$ c.p.s., we obtain $\Omega_0 = 958 \text{ rad s}^{-1}$, corresponding to a rotation period $P_0 = 6.6$ ms for the neutron star, and $a \approx 745$, which gives $B \approx 5.5 \times 10^9$ G (see Table 1). These are, indeed, typical values for millisecond pulsars^{2,6} spun up by accretion, particularly for those born in bright galactic bulge sources⁶. Note that if the observed frequency is actually the n th harmonic of the fundamental beat frequency²⁰ then the inferred Ω_0 values would be n times smaller, r_A values $n^{2/3}$ times larger and B fields $n^{7/6}$ times larger than the values given in Table 1.

From equation (5), we further find that

$$\alpha = \frac{d \log f}{d \log I} = \frac{d \log \omega}{d \log I} = \frac{3}{7} \frac{1}{1 - \Omega_0/a I^{3/7}} \quad (6)$$

α has the values 2.06 at $I = 3,100$ c.p.s. and 3.70 at $I = 2,400$ c.p.s. The best fit with a constant $\bar{\alpha} \equiv d \log \omega / d \log I$ to the $\omega(I)$ curve, given by equation (5), yields $\bar{\alpha} = 2.68$, in very good agreement with the reported value of ~ 2.5 . Indeed, this is true for any model with a beat frequency given by $\omega = a I^x - \Omega_0$ for $0 \leq x \leq 2.7$. Fitting such models in the observed range 2,400 c.p.s. $\leq I \leq 3,100$ c.p.s., one obtains, for each x , a range of $\alpha(I)$ between ~ 2 and ~ 4 ; this range narrows with increasing x , reaching the single value $\alpha = \bar{\alpha} = 2.7$ at $x = 2.7$, which is the limiting case where $\Omega_0 = 0$ (the neutron star is not rotating).

It is encouraging to find that a whole class of 'kinematic' models that take the observed quasi-period to be beat frequencies, with any rotation rate for the neutron star, can fit the observed α , provided that Ω_K increases with increasing $\dot{M}$. Furthermore, the particular dependence of r_A and of Ω_K on $\dot{M}$, given in equations (1) and (2), or something close to that, must hold very generally¹², as should the relation $L \sim G\dot{M}\dot{M}/R$. If, for example, the observed X rays originated at the instantaneous Alfvén radius, we would have $L \sim G\dot{M}\dot{M}/r_A$. This gives an exponent $x = 1/3$, instead of $3/7$, in equation (5), which then yields $\Omega_0 = 1,285 \text{ rad s}^{-1}$, $P_0 \approx 4.9$ ms and $B \approx 3.6 \times 10^9$ G, values not very different from those obtained with $x = 3/7$. Indeed, all models with $0 \leq x \leq 0.6$ give equilibrium rotation periods $P_0 < 10$ ms for the neutron star. Note that the kinematic model requires $\Omega_K(r_A) > \Omega_0 \equiv \Omega_K(r_A^0)$; hence, the instantaneous Alfvén radii r_A must have been closer to the star than r_A^0 , and $\dot{M}$ must have been larger than $\dot{M}_0$ throughout the observations of the quasi-periodicities in GX 5-1; otherwise, the beat frequency would have been $\omega = \Omega_0 - \Omega_K(r_A^0)$, and negative values of the index α would have been observed.

As an example of a type of physical mechanism that would result in such beat frequencies, we propose a 'magnetic gate' scenario operating through magnetic matching between a field configuration tied to the matter rotating at keplerian velocity at the instantaneous inner boundary of the disk, r_A , and the field configuration co-rotating with the neutron star. We start from an analogy with recent findings concerning the geomagnetosphere and the solar wind. Empirical correlations between magnetic storm and substorm activity in the Earth's magnetosphere

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and the energy flux from the solar wind entering the magnetosphere were established by Akasofu and Perreault¹³⁻¹⁵. They find that, among many observable parameters of the solar wind, the quantity that correlates with the rate of energy dissipation $u(t)$ in magnetospheric storms is the energy flux into the magnetosphere from the solar wind, $\varepsilon(t)$,

$$\varepsilon(t) = VB^2 L_0^2 \sin^4 \frac{\theta}{2} \quad (7)$$

where V is the wind speed, B is the solar wind magnetic field magnitude at the front of the terrestrial magnetosphere, located at radius L_0 , and θ is the local angle between the solar wind magnetic field and the Earth's magnetic field at that boundary. In equation (7), $L_0^2 \sin^4 \theta/2$ is the effective area through which the external flux enters the magnetosphere. Thus, the flux entering the Earth's magnetosphere is maximum at locations on the boundary where $\theta = \pi$ (ref. 16), where the rate of field line reconnection is presumably maximum. Moreover, the time dependence of this behaviour is not smeared by motions of the plasma. The material flow will thus be enhanced at $\theta = \pi$, and the flow pattern will be modulated by the instantaneous distribution of θ . In particular, if the two flows are simple rotations at different rates, the pattern of relative angles θ will rotate at their frequency difference, and the energy dissipation will be modulated accordingly.

To apply these ideas to the accretion disk, we first note that the energy flux arriving at the boundary is determined by the mass transfer through the keplerian disk. Close to r_A (within a thin boundary region^{17,18}), disk matter is close to pressure balance with the magnetospheric field. Taking $V \cong V_K(r_A) \equiv V_A$, $B^2 \cong 4\pi\rho V_A^2$, $L_0 = r_A$ and $4\pi r_A^2 \beta \rho V_A = \dot{M}$, where β is a dimensionless parameter reflecting the geometry of accretion across the Alfvén surface and ρ is the density of the matter, equation (7) gives

$$L(r_A(t)) = \frac{\sin^4 \frac{\theta}{2}(t)}{\beta} \frac{GMM(t)}{r_A(t)} \quad (8)$$

An average of this quantity over the boundary will determine the influx of matter into the magnetosphere. Thus the observed luminosity will contain some modulation at the beat frequency due to the time dependence in equation (8), through

$$\sin^4 \frac{\theta}{2} = \frac{3}{8} - \frac{1}{2} \cos \theta + \frac{1}{8} \cos 2\theta$$

We now postulate (see ref. 20): (1) that there is some well-defined magnetic field pattern at the boundary r_A^0 ; (2) that this pattern is fixed with respect to the matter rotating at $\Omega_K(r_A^0)$; (3) that it has some finite width Δr around r_A^0 , which we shall call the coherence length. For a non-aligned rotator, θ will generally depend on the azimuthal angle ϕ about the rotation axis of the star and the disk (assumed to coincide), and the only time dependence is rotation at $\Omega_K(r_A^0) = \Omega_0$. As $\dot{M}$ increases and r_A moves inward, pieces of the field pattern move in with the matter and adopt the new keplerian rotation rate $\Omega_K(r_A)$, without substantial smearing. We now have a pattern of θ which is no longer co-rotating with the magnetosphere: the pattern is now rotating at the beat frequency $\Omega_K(r_A) - \Omega_0$ with respect to the magnetospheric field. Hence, a modulation in accretion rate at the beat frequency (or some multiple of it) will appear. This state will be clearly distinct from equilibrium (at r_A^0) once r_A has moved in from r_A^0 by a few Δr . Furthermore, there should also be another memory effect—as r_A moves even further in, the field distribution carried by the matter should lose its coherence altogether.

The above picture can also explain the observed behaviour of the amplitude of the quasi-periodicity. At the lowest frequency observed (that is, when r_A is closest to $r_A^0 > r_A$), 8% of the received flux is quasi-periodic; however, the quasi-periodic fraction decreases with frequency until, at the highest observed frequency (r_A furthest from r_A^0), the quasi-periodic behaviour disappears altogether. We may estimate Δr from the observed

full width at half maximum (FWHM) in the power spectra displaying the quasi-periodicity: $\Delta\omega/\omega \sim 25\%$ gives $\Delta r/r_A^0 \cong 2/3 \Delta\omega/\omega [1 - (r_A/r_A^0)^{3/2}] (r_A/r_A^0) \cong (2-3\%)$. This is consistent with our estimates of the maximum and minimum Alfvén radii involved: $(r_A^0 - r_A^{\max})/r_A^0 \cong 8\%$ and $(r_A^0 - r_A^{\min})/r_A^0 \cong 14\%$ (see Table 1). A coherence time scale for the field pattern to follow radial adjustments to r_A can also be defined as $\tau \equiv \Delta r/V_r$, where V_r is the radial velocity of matter through the disk. This time scale τ must clearly be small compared with the time scale of $\dot{M}$ variations. τ is a measure of the decay time (in the radial direction) of the magnetic field pattern. Thus, if our interpretation of the observation is correct, the observed FWHM $\Delta\omega$ provides a clue to this decay time. Note that it is the instantaneous value of $\dot{M}$ at r_A which gives the correlation; as matter falls in from there rather rapidly, no delay in the correlation is expected.

It is not clear whether the present observations reflect an episode of increasing $\dot{M}$ alone or whether, in the case of decreasing $\dot{M}$ (r_A increasing from r_A^0), the field pattern does not survive coherently. We emphasize that decreasing $\dot{M}$ would give negative values of α , so that observations of negative α would indicate variations in both directions from the equilibrium configuration at r_A^0 . Note that GX5-1 may still be, on the average, spinning up.

One particularly attractive model for realizing the topological discontinuities needed for having the beat-frequency effect is, simply, accretion of magnetized clumps of matter²⁰. As each clump moves across the co-rotation ring at r_A^0 to r_A , it is absorbed in the magnetosphere at the beat rate; absorption of N incoherent clumps only reduces the modulation by $\sqrt{N}$. A logical consequence from such model is red frequency noise in the accretion²⁰. Note, that obscuration and inobservability of the fundamental stellar pulsations at Ω_0 may be here simply due to a thick disk²⁰.

The problems of magnetosphere accretion disk interaction have been discussed extensively (see, for example, refs 12, 17, 18, 19 and references therein). Solutions to particular cases and the difficulties and special assumptions involved testify to the complexity of the problem. Examples of other physical interaction to make the beat frequency observable are discussed in ref. 20 (M.A.A., F. K. Lamb, J.S. and N. Shibazaki, in preparation). On the observational side, further observations of GX5-1 and other galactic bulge sources are of great interest. An observation of a rotation period in the millisecond range as predicted would provide strong support for our kinematic model, but may be elusive in view of the absence so far of any detection of a rotation period and in view of the theoretical picture of galactic bulge sources²¹. One set of past observations, those of the 1,000-s quasi-periodicity in the 8-s pulsating source 4U1626-67²², as well as the 0.5-s quasi-periodicity in the rapid burster²³ and the quasi-periodicity in SCO X-1 (J. Middleditch and W. Friedhorsky, personal communication) may be possible candidates for similar beat frequency effects.

The particular mechanism described above for modulations at the beat frequency may not be unique. A search for similar effects in slow rotators and in massive X-ray sources may give further clues to the differences in the accretion process between these different classes of X-ray sources and will assist in sorting out other possible physical models.

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Onset of sublimation in comet P/Halley (1982i)

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We report here the first direct evidence for the onset of sublimation of a comet nucleus. Emission due to CN observed in spectra of comet P/Halley (1982i) provides evidence for the development of the gas coma. Broad-band photometric observations of the comet indicate that the dust coma developed near a pre-perihelion heliocentric distance, $r \sim 6$ AU. We have derived rates of gas production and brightening for comet P/Halley at $r \sim 4-6$ AU and use the mean pre-perihelion nuclear magnitude derived for the comet ($r > 8$ AU) to calculate an effective radius of the nucleus (R), which for plausible values of the geometric albedo, $p_v \sim 0.03-0.6$, lies in the range $R = 1-4$ km.

Pre-perihelion spectrophotometric observations (wavelength $\lambda = 3,000-7,000$ Å, $\lambda/\Delta\lambda \sim 250$) covering a heliocentric distance (r) in the range 5.6-4.2 AU of comet P/Halley were obtained using the photon-counting Reticon spectrograph on the 4.5-m Multiple Mirror Telescope (MMT) of the F. L. Whipple Observatory (Table 1). A ratio of the spectrum of comet P/Halley with the solar spectrum is shown in Fig. 1, which provides direct evidence for a gas coma in the comet. The presence of the CN emission band (3,883 Å) is evident in the comet spectrum, confirming our previous report of detection of CN emission in the 17 February 1985 MMT data¹.

All the MMT spectra listed in Table 1 have been ratioed and differenced both with the solar spectrum² and with that of a solar analogue to search for weak cometary emission features. Comparison of the February and March spectra of comet P/Halley indicates that the emission feature at 3,883 Å is the only feature present above the 1.5σ detection level in both spectra at the same wavelength and with a width larger than one resolution element. No statistically significant detection of the CN band was found in the November 1984 spectrum. Only a marginal detection of CN emission was indicated in the April spectrum, which may be explained by the fact that the comet was observed through a considerable air mass and for a shorter integration time than in February and March. No other cometary emission features would be expected to be detectable.

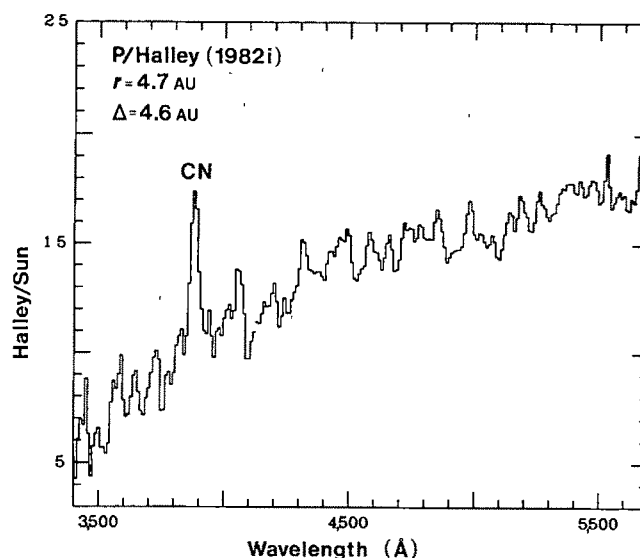


Fig. 1 Detection of the $\Delta v = 0$ band sequence of the violet CN system ($B^2\Sigma^+ - X^2\Sigma^+$) is illustrated in this averaged (February and March data) pre-perihelion MMT spectrum of P/Halley (1982i) which has been ratioed with the solar spectrum. The CN emission demonstrates the presence of a significant gas coma in the comet. This spectrum was binned in 10-Å intervals before division by the solar spectrum, which was also binned in 10-Å intervals. Note the colour difference, $\Delta(B - V) \sim +0.2$, in the sense (P/Halley-Sun) indicated by the slope of the ratioed continua.

Table 1 shows measurements and 2σ upper limits for the CN emission band fluxes obtained from the (Halley-Sun) difference spectra after normalization at the continuum near 3,883 Å. The CN band fluxes were converted to column densities, and CN production rates were calculated using the vectorial model³. In the calculation, photo-dissociation lifetimes ($r = 1$ AU) of 22,000 and 100,000 s were adopted for the CN parent and for CN, respectively. The assumed parent velocity was 0.8 km s⁻¹ and that for CN 3.0 km s⁻¹. Assuming $Q(\text{OH})/Q(\text{CN}) \sim 500$, the production rate of H₂O in P/Halley at $r \sim 4.7$ AU is estimated to be $Q(\text{H}_2\text{O}) \sim 2 \times 10^{27}$ molecules s⁻¹.

Photometric data derived from the continua of the MMT spectra, together with published broad-band photometry of comet P/Halley, provide evidence for the onset of sublimation of the nucleus. To our knowledge this represents the first observation of the onset of sublimation in any comet. The V magnitudes, uncorrected for aperture effects and derived by convolving the MMT spectra with the standard V passband transmission function⁴, are listed in Table 1. The comet was visible on the MMT television monitor during the February, March and April observations. In March and April the average seeing diameter was $\sim 1-2$ arc s and the images of P/Halley had soft appearances relative to stars in the same field, indicative of a resolved cometary image (angular diameter > 2 arc s). During these observations the comet was not seen on the television monitor more extended than 5 arc s to a surface brightness level of $V \sim 21-22$ mag arc s⁻². The spectrum in November was obtained using a blind offset, as the comet was too faint to be seen on the television monitor.

Table 1 MMT Reticon observations of P/Halley (1982i)

Date (UT)	r (AU)	Δ (AU)	Φ °	Aperture* (km)	$V^\dagger$ (mag)	$V(1,0)$ (mag)	$F(\text{CN})$ (erg cm ⁻² s ⁻¹)	$Q(\text{CN})$ (molecules s ⁻¹)
26.4 November 1984	5.60	4.76	5.6°	8,850	20.9	13.6	$< 1 \times 10^{-15}$	$< 6 \times 10^{25}$
17.2 February 1985	4.84	4.45	11.2°	8,050	19.9	12.8	$(3 \pm 2) \times 10^{-16}$	$(3 \pm 2) \times 10^{24}$
24.2 March 1985	4.50	4.73	12.1°	8,600	19.4	12.4	$(5 \pm 4) \times 10^{-16}$	$(4 \pm 3) \times 10^{24}$
18.2 April 1985	4.25	4.87	10.0°	8,850	18.7	11.8	$< 2 \times 10^{-15}$	$< 1 \times 10^{25}$

* Projected circle equivalent radius of 2×3 arc s aperture in November; projected radius of 5 arc s diameter circular aperture in February, March and April.

† Estimated internal error is ± 0.3 mag. Both magnitude and flux measurements for February data supercede those given in a preliminary report¹.

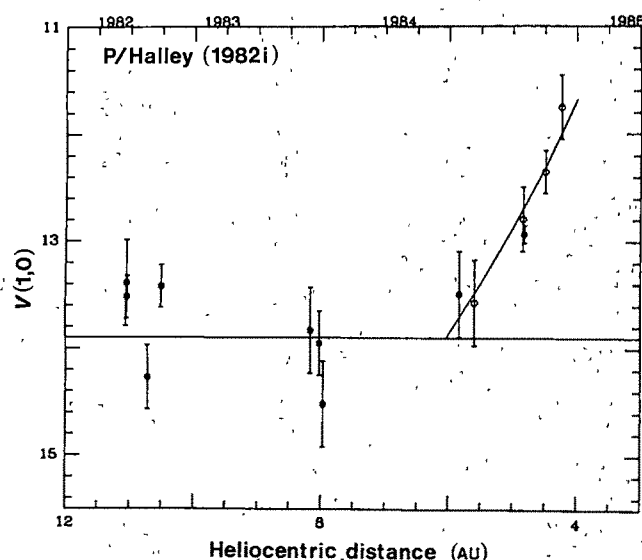


Fig. 2 Evidence for the onset of sublimation of the nucleus of comet P/Halley near $r \sim 6$ AU. $V(1,0)$ is the V magnitude of the comet reduced to a distance 1 AU from the Earth, 1 AU from the Sun and to a direction directly opposite the Sun. An inactive comet nucleus would have a constant $V(1,0)$ magnitude. Dates at the top represent mid-year epochs.

To remove brightness variations resulting from changes in the heliocentric and geocentric distances of the comet, the V magnitudes were converted to reduced magnitudes using

$$V(1,0) = V - 5 \log r - 5 \log \Delta - 0.039 \Phi \quad (1)$$

where $V(1,0)$ is the magnitude of the comet at $r = \Delta = 1$ AU, V is the observed magnitude (Table 1), Φ is the solar phase angle, and an asteroid phase function, 0.039, is assumed. In Fig. 2 the reduced magnitudes of P/Halley are plotted against the heliocentric distance; photometry published elsewhere³⁻¹¹ has also been included. An inactive cometary nucleus would be expected to have a constant $V(1,0)$ (except for the effects of rotation and aspect).

There is clear evidence for an increase in the brightness of the comet at $r \sim 6$ AU, indicative of the development of a dust coma (see Fig. 2). Significant continuous sublimation of the nucleus of P/Halley commenced sometime between mid-1984 and early 1985 (Fig. 2). This onset epoch is consistent with the observation of an extended coma reported in late September 1984 by Djorgovski and Spinrad¹². In fact the September appearance of the extended coma associated with comet P/Halley could have resulted from a rupture of a surface dust mantle because of solar heating after the 75-yr incubation period of the comet. Note that the lack of CN in the November spectrum (Table 1) does not preclude the estimated sublimation onset epoch, as CN emission would have been undetectable (at the 1.5σ level) with the MMT Reticon spectrograph even if the November band strength had been comparable with that observed in February 1985.

The constancy of the mean value of the reduced magnitude for $r > 8$ AU indicates that systematic sublimation was not occurring in comet P/Halley from $r \sim 12$ to 8 AU. Hence, we conclude that the observations obtained when the comet was located at $r > 8$ AU represent the inert nucleus. For $r > 8$ AU we calculate the mean reduced nuclear magnitude of the comet, $\overline{V(1,0)}$, to be 13.9 ± 0.5 mag, where the error represents the r.m.s. deviation in the data. The intersection of the sublimation curve with the $\overline{V(1,0)} = 13.9$ line in Fig. 2 gives a heliocentric distance corresponding to the onset epoch, $r \sim 6 \pm 1$ AU.

The photometric observations of comet P/Halley are plotted as a function of heliocentric distance in Fig. 3. The secular variation of the nuclear magnitude of comet P/Halley with heliocentric distance can be explained adequately by changes in heliocentric and geocentric distances and phase angle [equation (1)] during the interval 1982-85 ($r = 12-6$ AU). A

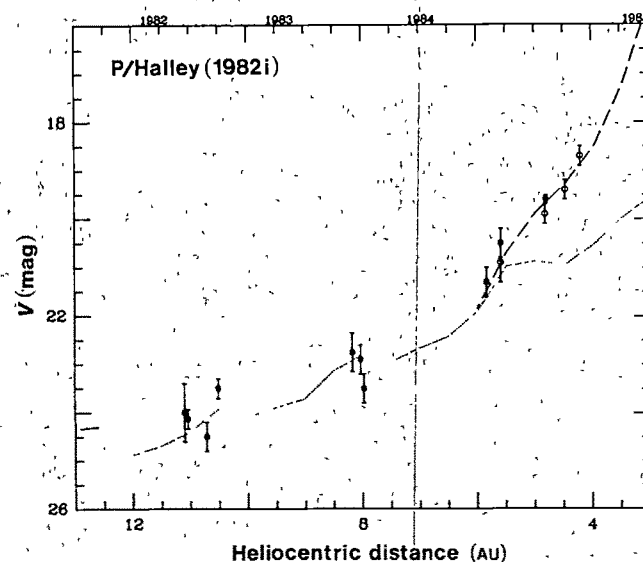


Fig. 3 Light curve of comet P/Halley (1982-85) showing the predicted nuclear magnitude (equation 2; dotted line) and the predicted total magnitude ($V \sim r^{-7 \pm 3}$; dashed line). The secular increase in brightness of comet P/Halley can be explained within the observational uncertainties by relative changes in distance (and phase angle) between the Earth, Sun and comet until the comet reached a heliocentric distance of $r \sim 6$ AU, when systematic brightening indicative of the dust coma development.

curve fitted to the photometric observations after the onset of sublimation indicates an initial dependence of the sublimation rate $V \sim r^{-7 \pm 3}$.

Figures 2 and 3 show that the photometric data obtained from comet P/Halley before $r \sim 8$ AU probably refer to the bare nucleus. Hence, an effective radius can be calculated using the mean reduced magnitude, from

$$p_v R^2 = k 10^{(0.4[V(\odot) - \overline{V(1,0)}])} \quad (2)$$

where $k = (1.5 \times 10^8 \text{ km})^2 / \pi$, p_v is the geometric albedo ($\lambda \sim 5,500 \text{ \AA}$), R is the effective (circle equivalent) radius of the nucleus, $V(\odot)$ is the apparent visual magnitude of the Sun (-26.7) and $\overline{V(1,0)}$ is the mean reduced magnitude of the comet nucleus, 13.9, derived above. We find for comet P/Halley

$$p_v R^2 = 0.5 \pm 0.2 \text{ km}^2 \quad (3)$$

which differ slightly from a previous determination by Hughes¹³.

As the albedo of the comet nucleus is not yet known, we adopt a plausible range of albedos, $p_v = 0.03-0.6$, which spans low-albedo asteroids to the higher-albedo icy surfaces such as the jovian satellites. For this albedo range, we calculate an effective radius for the nucleus of comet P/Halley, $R = 1-4 \text{ km}$. Note that if there is a significant contribution to the $\overline{V(1,0)}$ magnitude from a particulate halo surrounding the nucleus, then this range represents an upper limit to the effective radius of the solid-body nucleus of comet P/Halley.

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Interplanetary conditions during 3-kHz radio-wave detections in the outer heliosphere

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A very important observation¹ regarding the plasma physics of the outer heliosphere has been that plasma-wave instruments on both the Voyager 1 and 2 spacecraft beyond ~12 AU detected, during the same interval of time, plasma waves (frequency ~3 kHz) that could possibly be associated with the turbulence expected at the heliopause. It was suggested that this turbulence had propagated inwards to the spacecraft, which would place the heliosphere boundary at ~46 AU. To interpret these unusual observations, it is important to understand the characteristics of the interplanetary medium at large heliocentric distances. We draw attention here to the low-energy charged-particle environment in the outer heliosphere during the observations of the unusual plasma-wave signals. These particle data suggest that the outer heliosphere was unusually stable and free of transient shock and particle events for the ~8 months during the wave observations.

Figure 1 shows the counting rates as a function of time for protons (~3–17 MeV) measured by the Low Energy Charged Particle (LECP) experiment² on both Voyager spacecraft from 1982 to 1984. The plotted data are 3-day averages. The dotted line on Fig. 1 is day 243, 1983, when the unusual plasma waves were first observed¹. The wave signals are reported to have disappeared in early spring 1984 (ref. 3). During this interval of 1983–84 (which we subsequently refer to as the signal period, SP) the two Voyager spacecraft were at approximately the same helio-longitude, with Voyager 1 increasing in helio-latitude from ~20.5° to ~22.5° and in distance from ~17.2 to ~19 AU while Voyager 2 remained approximately in the ecliptic plane, moving from ~12.5 to 13.7 AU (ref. 1) on its way towards encounter with Uranus in January 1986.

During the SP interval, Voyager 1 measured no energetic protons for about 200 days. Before day 243, 1983, and after about day 75, 1984, many transient proton enhancements were observed. Voyager 2, in the ecliptic plane, observed some proton activity at about day 270, but, in general, the activity in the SP interval was much suppressed compared with that before day

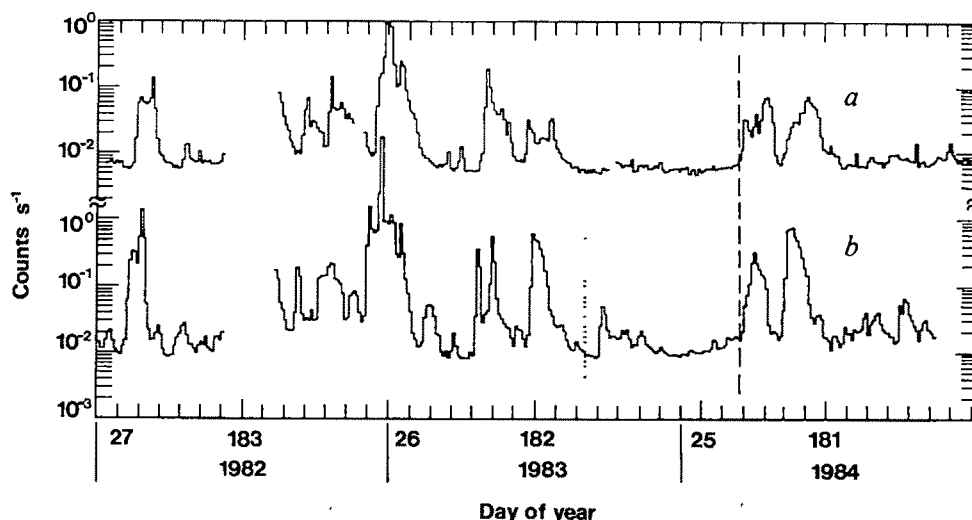
243, 1983, and after day 75, 1984. Examination of Voyager LECP ion data (~50 keV) shows that the only activity during the SP interval was from co-rotating interplanetary shocks⁴. This conclusion was also reached by the Voyager cosmic-ray instrument team studying >0.5-MeV protons⁵.

We suggest that this ~200-day interval of quiescence in the energetic ion population in the outer heliosphere was directly related to the fact that the anomalous plasma waves (waves at frequencies above the local plasma frequency in all cases) were observed. We suggest that the interplanetary medium at this time consisted only of outwardly propagating co-rotating shocks, providing a rather stable interplanetary environment for the propagation (and possibly generation) of plasma waves. No transient shocks from solar flares overtook the co-rotating shocks to disrupt the stability of the medium. Indeed, the medium may well have resembled, for ~200 days, the prediction of Burlaga⁶, who speculated that beyond ~10 AU the large-scale dynamics of the interplanetary medium could be dominated by pressure waves; the originating coronal signatures would be lost. The solar wind would, therefore, be more homogeneous on a large scale. We suggest that the dashed line in Fig. 1 marks the end of the wave observations in early spring³.

The basic question then arises as to the origins of the plasma waves detected during this interval by the two Voyager spacecraft. We note that in a stable medium consisting only of co-rotating shocks, the plasma waves, if generated at the plasma frequency at the heliosphere boundary, could propagate to the spacecraft in a medium undisturbed by disrupting transient events. The propagation could be across the shocks or along the spiral field configuration between the shocks. Further, the absence of energetic protons at both spacecraft for such a long interval implies the absence of such protons at the heliopause. Such energetic protons in association with transient shocks, in the turbulence of the heliopause, could disrupt the plasma-wave generation processes there. Alternatively, the observations of the unusual plasma waves during this time interval could arise from plasma-wave generation at about twice the local plasma frequency, at the co-rotating shocks, with the waves channelled preferentially between the shock structures in the unusually stable interplanetary conditions.

While considerable study and modelling have been reported on the large-scale magnetic character of the inner heliosphere^{7–10}, less work has been reported on the heliosphere structure beyond ~10 AU and, in particular, on the propagation of heliosphere plasma waves in the various magnetic structures formed by co-rotating shocks and/or by transient shock conditions. The possibility that the heliopause has been remotely detected during the stable interplanetary conditions reported here should stimulate work on studies of the propagation of plasma waves in the outer heliosphere in an ordered

Fig. 1 Proton counting rates (3-day averages) measured on the Voyager 1 (a; 3.4–17.6 MeV) and Voyager 2 (b; 3.0–17.3 MeV) spacecraft from 1982 to 1984. The dotted line marks the onset of 3-kHz radio-wave observations by Voyager¹. The dashed line marks the expected termination of the detection of the waves determined from considerations of the energetic ion fluxes in the outer heliosphere.



interplanetary environment devoid of transient shocks and energetic ions.

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Characteristics of *in situ* coal gasification liquids from a cretaceous coal

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The concept of harnessing energy from fossil fuel by gasification of coal in the ground has been revived with the aim to increase usable coal reserves and to minimize the environmental impacts of mining and other problems. We report here the characteristics of liquids formed as a by-product of an underground gasification pilot test burn of a Cretaceous coal seam at a central Alberta location. The simplicity of the composition of liquids produced suggests that *in situ* coal gasification may be an economically viable source of refinery feeds or fuel.

During the summer and early autumn of 1976, an exploratory field test of underground coal gasification was carried out in the Battle River coal field, ~195 km south-east of Edmonton, on a 3.6-m zone of Cretaceous sub-bituminous coal. This coal zone is part of the Horseshoe Canyon Formation and lies almost horizontally under ~18 m of fairly impervious shale and clay. A rectangular 18.3 × 9.1 m four-well system was placed in the direction of the face cleat to facilitate the breaking of the coal according to its natural trend. The boreholes were so linked by surface piping that any one hole could serve for air injection or for delivery of gas through a knock-out tank (for stripping tarry materials from the production gas) to the flare stack. During the gasification segment of the test burn, ~300 tonnes of coal were affected and 25,500 m³ of gas with a heating value of 3,700 kJ m⁻³ produced. Excavation of the site after the test burn indicated that 50 tonnes of dry coal were pyrolysed and gasified. A substantial quantity of tar and light oil was collected at the knock-out tank while the gasification was in progress. The simplicity of composition of these liquids suggests that by-products of *in situ* coal gasification may be an economically viable source of refinery feeds or fuel.

To provide laboratory support for the field test programme, we designed a simulator which used a 1 × 1 × 2 m coal block retaining most of the essential features of an undisturbed coal seam, and several simulations of underground coal gasification (UCG) were performed. Detailed descriptions of the simulation experiments¹ and field tests²⁻⁴ have been published elsewhere.

An insight into the composition of the organic content of the liquid effluents from the field tests was gained by liquid-liquid extraction of an aliquot of the product sample with an organic solvent, followed by IR spectrometry on the extract. The spectra, showing the presence of strong —OH and —O— bands, together with well-resolved 1,600–1,800 cm⁻¹ bands, were almost identical to spectra obtained for low-temperature pyrolysis tar of

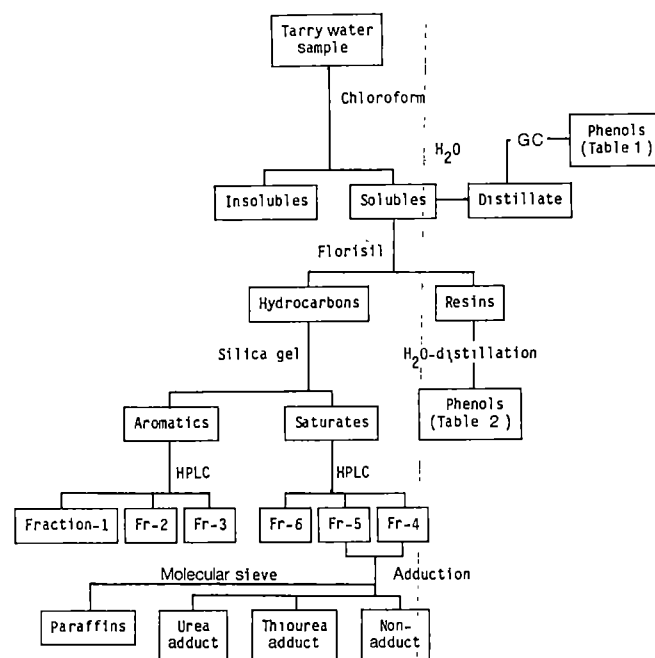


Fig. 1 UCG liquids separation scheme.

low-rank coals. As the IR spectra of field and laboratory test samples indicated the presence of carboxylic acid and acid anhydrides, more detailed analyses of the liquid effluents collected as spot samples from the slipstream of the product line, from the knock-out tank and from the slipstream before the flare stack, were carried out as the field test progressed.

The final separation scheme (Fig. 1) was as follows: the samples were exhaustively extracted with chloroform; the solids were removed by filtration, and after removing chloroform from the filtered chloroform layer, a fraction of the residuum was separated into 'hydrocarbon' and 'resin' fractions by elution chromatography on a Florisil column. The resin fraction was steam-distilled and the steam-volatile fraction analysed for phenols by gas chromatography. The hydrocarbon fraction was separated on silica gel to give 'saturates' and 'aromatics', and each of these was re-separated by HPLC on amino phase (Lichrosorb-NH₂) using *n*-hexane eluent. HPLC separation showed that the 'saturates' from silica gel were contaminated with a small amount of 'aromatics' and these were removed as HPLC fraction 6. The HPLC 'saturates' fractions 4 and 5 were combined and further adducted with urea and thiourea to give urea-adduct, thiourea-adduct and thiourea-nonadduct following standard procedures⁵⁻⁷. Part of the 'saturates' was adducted on a molecular sieve (5 Å).

A large proportion of the steam-volatile fraction was identified as phenols (Table 1). The first member (phenol) of the homologous series was by far the most abundant constituent, followed by cresols (meta-, *m*-; para-, *p*-; and ortho-, *o*- in that order), while the remaining higher phenols were present only in minor proportion. The distribution of phenolic compounds remained constant during the steady burn period. A build-up of trimethyl phenols was noted in the product stream. Between 20 and 25% of total phenols escaping into the flare stack were identified as trimethylphenols on the fourth day of the burn period (sample F-4-3 and F-4-3₂).

The relative concentrations of the classes of compounds separated on the Florisil and silica column are shown in Table 2. The percentages of the chloroform-soluble liquids from effluents in steady burn conditions were 30% saturates, 25% aromatics, and 19% phenols; complex phenols plus compounds of high relative molecular mass (noted as non-volatile resin, Table 2) made up the last 26%. The error of analyses was ±3–5%. The hydrocarbons representing >50% of the UCG liquids were separated into saturates and aromatics on silica gel, and into six fractions

Table 1 UCG field test: phenol contents and distribution in liquid product effluent

Sample	F-1-1	F-1-2	F-4-3	F-4-3*	F-4-3 ₂	F-4-3 ₂ *
Wt % organics						
Steam-volatile fraction	50.0	68.3	15.0		15.0	
Phenols	32.9	55.3	8.3		7.8	
% Composition of phenols						
Phenol	43.0	45.0	13.0	13.8	13.1	14.2
<i>o</i> -Cresol	7.6	6.8	5.5	5.5	5.2	2.9
<i>m</i> -Cresol	20.7	20.6	14.3	15.8	14.2	17.0
<i>o</i> -Ethylphenol	0.7	0.5	1.5	1.5	1.3	0.4
<i>p</i> -Cresol	9.2	9.5	9.3	11.0	8.4	11.0
<i>m</i> -Ethylphenol	2.2	2.1	3.1	3.4	3.3	4.4
<i>p</i> -Ethylphenol	2.6	2.4	5.5	5.2	4.9	6.7
Dimethylphenol 2,6-	0.6	0.1	1.9	1.3	2.0	0.9
2,4- and 2,5-	3.2	2.2	8.6	7.9	7.6	3.9
2,3- and 3,5-	5.2	4.6	8.1	7.2	8.0	8.7
3,4-	3.3	2.4	6.0	4.7	7.0	7.4
Trimethylphenol 2,4,6-	2.3	2.2	8.9	3.3	10.7	10.0
2,3,6-	?	1.6	11.2	17.8	13.0	10.1
2,3,5- and 2,4,5-	?	?	3.2	1.4	1.1	2.4

* Phenols separated by carbonate wash. F-1-1, sample from day-1/slipstream before knock-out (KO) tank; F-1-2, sample from day-1/KO tank; F-4-3₂, sample from 2nd test day-4/slipstream after KO tank; F-4-3₂, sample from day-4/slipstream after KO tank (1st test)

by HPLC. Preliminary characterization of the HPLC aromatic fractions came from a comparison with the retention times of a standard aromatic hydrocarbon mixture (benzene, naphthalene and phenanthrene); this suggested that fraction 1 consisted of mono- and di-aromatic hydrocarbons, fraction 6 mostly di-aromatic, and fraction 2 contained di- and tri-aromatic hydrocarbons only. More complex materials were present in fraction 3. Probable structures and compositions of all separated fractions were derived from subsequent analysis by gas chromatography and mass spectrometry (GC-MS).

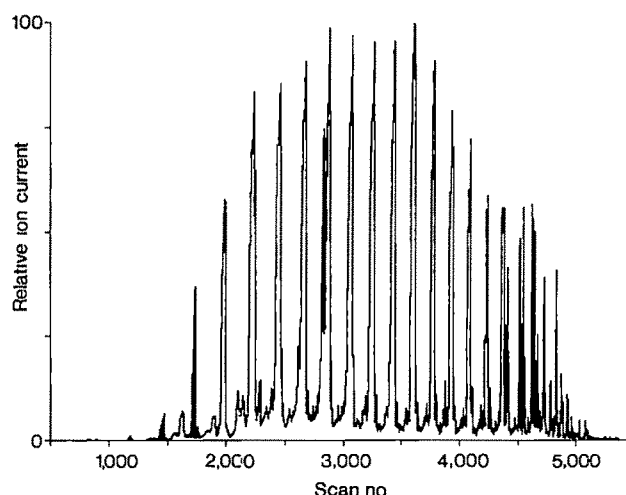
The first major peak in fraction 1 was identified as naphthalene by GC-MS analysis. Subsequent peaks were methyl, dimethyl and trimethylnaphthalenes. Also, in the same chromatogram, a series of minor peaks can be observed between scan 2,000 and 3,000 belonging to acenaphthenes and possibly biphenyls. Fraction 2 seemed to be an intermediate zone between di- and poly-aromatics. Here the major group of compounds were dibenzofurans with minor amounts of acenaphthenes and biphenyls. The series of molecular ions M^+ 168, 182, and so on, represented dibenzofurans, while the isobaric acenaphthenes were concentrated in fraction 1. The higher members of the homologous series were the corresponding di- and trimethyl derivatives.

Fraction 3 was spectroscopically resolved into two groups. The first group was identified as anthracenes-phenanthrenes from the first member of the series to their tetramethyl homologues. No attempt was made to distinguish between anthracenes and phenanthrenes. The second group has the two largest peaks represented by pyrene and fluoranthene, together with smaller amounts of their monomethyl homologues. Careful extraction of mass spectral data for scan 3,500 and 4,000 indicated that a smaller amount of chrysene and a minute quantity of perylene were also present in this fraction. The total ion count

Table 2 UCG tar composition according to separation scheme (wt % distribution)

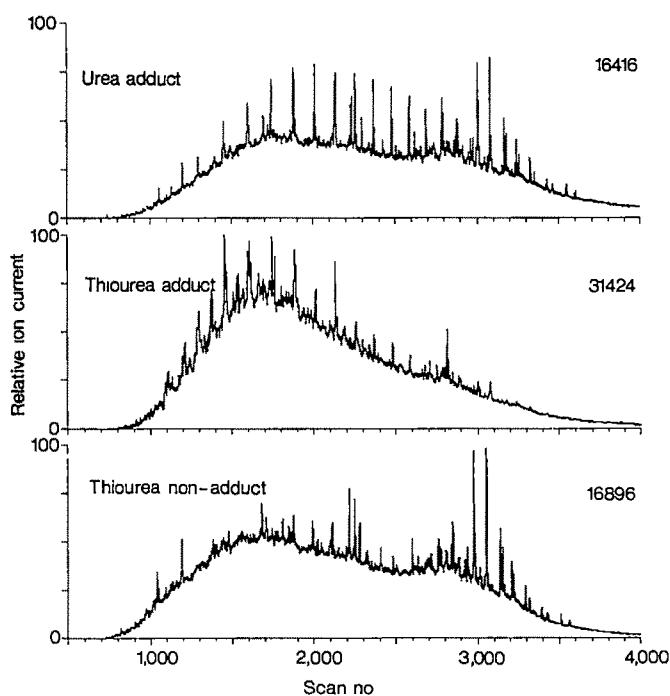
Sample	F-3-1*	F-3-2*	F-4-3	F-4-3 ₂ (tar only)	F-4-3 ₂ (tar in water)
Particulates					
+asphaltic matter	12.5	6.6	3.7	5.3	4.3
Saturates	31.2	26.6	19.4	28.1	23.6
Aromatics	18.7	16.6	20.2	30.4	28.5
Steam-volatile resin	12.5	26.6	11.7	10.7	26.8
Non-volatile resin	18.1	20.0	36.9	22.5	17.3
Loss	7.0	3.6	8.1	3.0	—

* F-3-1, sample from day-3/slipstream before KO tank; F-3-2, sample from day-3/KO tank.

**Fig. 2** Mass chromatogram of saturate fractions.

(TIC) chromatograms provided an approximate distribution pattern, the relative order of abundance being anthracene-phenanthrene followed by pyrene-fluoranthene, di- and tetramethyl naphthalenes, methylnaphthalene, acenaphthene/biphenyl, dibenzofurans, chrysene and acenaphthenes.

Figure 2 is the TIC chromatogram of the combined saturate fractions 4 and 5. Here the peaks are equidistant; superimposed on them are two series of peaks—one at a lower scan and the other at a scan number from 4,100 to the end of the chromatogram. The identity of the group of major peaks was confirmed as a homologous series of *n*-paraffins. C_{14} – C_{24} paraffinic hydrocarbons were the main constituents. After the removal of paraffins on a molecular sieve, the sample was separated into urea and thiourea adducts and thiourea nonadduct (Fig. 3). The three fractions of adduction show, respectively, a wide distribution of very small amounts of open-chain to pentacyclic hydrocarbons. The presence of sterane and hopane hydrocarbons together with smaller amounts of tetra- and tricyclic hydrocarbons was identified from simple cross-scans of the thiourea nonadduct fraction combined with a consideration of the retention time. The retention times correspond to C_{21} – C_{29} steranes and C_{27} – C_{34} hopanes.

**Fig. 3** Mass chromatogram of urea and thiourea adducts and of thiourea nonadduct (number in upper right is total ion count).

Thus the composition and compound types present in the UCG liquid from a Cretaceous coal field were established. The paraffinic hydrocarbons, hopanes and steranes, along with aromatic hydrocarbons and phenols, represented >80% of all constituents. The abundance of the hydrocarbons was too high to discount them as coal seam contaminants; it is more likely that these are an integral part of the 'coal molecule' or are present in the coal as 'inclusion compounds'. Deno and co-workers⁹ obtained long-chain paraffinic acids by selective oxidation of various types of coals by trifluoroacetic acid oxidation. The simplicity of composition of the UCG liquids suggests the economic potential of this liquid for use as refinery feed or fuel.

A major omission of the evaluation of field tests and simulation experiments was the absence of quantification of liquid yield per tonne of coal. After excavating the test sites, it was observed that the gasification zones in the test burns always followed a pyrolysis zone containing disintegrated pyrolysed char retaining no trace of the cleat orientation. The temperature of this zone was below 600 °C as revealed by an analysis of the char⁴. It is then reasonable to assume that the UCG liquids were formed predominantly by pyrolytic reactions, and contained the most stable and steam-volatile components. An optimum estimate of liquid yield could thus be expected from the Fischer-Schrader assay of this coal. The 50 tonnes of dry coal (affected in the test burns) gave 15 tonnes of ASTM (American Standard for Testing Materials) volatiles which contained 5 tonnes of Fischer gas, 3 tonnes of product water and 7 tonnes of Fischer light oil and tar. The leftover carbon in the pyrolytic char was gasified to give 16 tonnes of carbon monoxide (~13,000 m³).

Steam-volatility of UCG liquids may cause contamination of the aquifer and create new ecological concerns. The close monitoring of groundwater around the Alberta test zone over a 2-yr period did not show any phenolic contamination to a detectable level in the groundwater. A continuous large-scale operation has to be monitored more thoroughly.

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Synthetic seismogram modelling of the oceanic P_n phase

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The seismic oceanic P_n phase is characterized by low spatial attenuation, a high-frequency wavetrain of extremely long duration and an onset velocity near 8.0 km s⁻¹. In an effort to explain these properties, models have been proposed for the mode of propagation of this phase which include energy entrapment within a low-velocity channel^{1,2}, propagation within an extremely low-attenuation lithosphere waveguide^{3,4} and forward-scattering from local heterogeneity within the oceanic crust^{5,6}. Through synthetic seismogram modelling, we have generated realistic oceanic P_n phases for an oceanic lithosphere model that did not contain any of these intrinsic properties. The synthetic P_n phase can be explained rather simply as a set of refractions from the lower lithosphere with

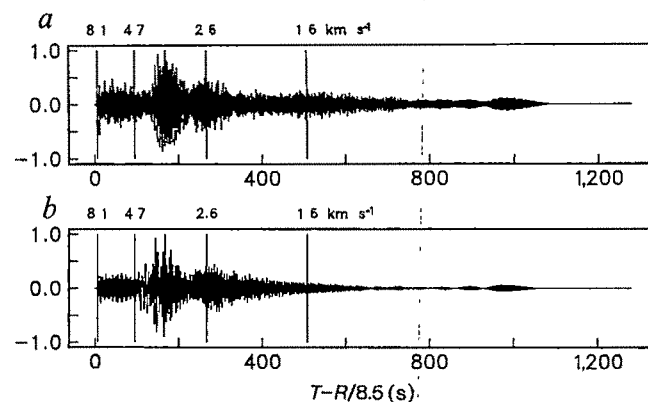


Fig. 1 Complete synthetic seismograms at an epicentral range, R , of 1,000 km for a thrust fault source at 14.0 km depth. The fault had a dip of 45°, a rake of 270° and an azimuth relative to the receiver of 60°. *a*, Vertical component; *b*, horizontal component rotated 25° clockwise from the radial direction. Group velocities of 8.1, 4.7, 2.6 and 1.6 km s⁻¹ are indicated. Phase velocities between 3.85 and 25.0 km s⁻¹ were included in the calculation.

subsequent reverberations within the oceanic water column and sediment layer. Many of the spectral properties associated with layer reverberation observed in the synthetic P_n phase are revealed in data collected in the south-west Pacific. Complicated oceanic lithosphere models are not required to explain the gross characteristics of P_n propagation.

The realistic oceanic P_n phases were synthesized using wavenumber integration⁷. The technique involves a quadrature over wavenumber at fixed frequency points with the result then Fourier transformed into the time domain. The resulting seismograms contain all reverberations, including those off the free surface, as well as all P to S (primary to secondary) and S to P mode conversions. Although the algorithm is similar to the widely used reflectivity method⁸, it differs in several important respects. The inability of reflectivity to incorporate reverberations and mode conversions outside a 'reflection zone' that cannot contain the free surface renders the method inadequate for the study of P_n propagation. We will show that near-receiver sediment and water reverberations are quite important in the synthesis of oceanic P_n wavetrains and, therefore, that wavenumber integration is well suited for the study of this phase.

The most serious limitation of wavenumber integration is the restriction to models consisting of a stack of laterally homogeneous layers. We have chosen a model representative of normal oceanic lithosphere, consisting of a vertically inhomogeneous oceanic crust and upper mantle overlain by a 4-km-thick water layer and a 500-m-thick sediment layer⁹. The values of the quality factor, Q , chosen in agreement with seismic refraction studies¹⁰, were <500 for compressional waves in the crust and upper mantle and 50,000 in the oceanic water column. A low-velocity channel was not included and the seismic velocity was a monotonically increasing function of depth.

Figure 1 shows complete synthetic seismograms generated for a sub-Moho thrust fault at an epicentral range of 1,000 km. The vertical component is illustrated in Fig. 1*a* and the horizontal component in Fig. 1*b*. The division of these seismograms into two groups facilitates the schematic identification with the oceanic P_n and S_n phases. The group with an onset velocity near 4.7 km s⁻¹ (Fig. 1*b*) is grossly identified as the oceanic S_n phase. The first-arriving energy within this group is entirely comprised of horizontally polarized shear waves and is absent from the vertical component. The absence of a clear SV body wave at a velocity near the mantle shear velocity, 4.7 km s⁻¹, may indicate that the mantle shear Q used to calculate the synthetics, 225, was too low. Work is continuing in an effort to synthesize more realistic S_n phases.

The first-arriving group of energy in Fig. 1 has an onset velocity of 8.1 km s⁻¹, a duration of the order of 100 s and extends into

the onset of S_n . This group is characteristic of the oceanic P_n phase.

The importance of reverberations within the oceanic water column and sediment layer is most effectively demonstrated in the frequency domain. It is simple to show that the power spectrum of a wavelet reverberating in a single layer contains spectral peaks at frequencies given by

$$f_n = (2n+1) \frac{df}{2}, \quad (n=0, 1, 2, \dots) \quad (1)$$

where $df=1/T$ and T is the time between multiples. In the case of near-vertical incidence, T can be approximated by the two-way travel time in the layer and equation (1) represents the eigenfrequencies for an organ-pipe mode¹¹. The restriction of P_n to rays with turning points in the sub-Moho portion of the oceanic lithosphere requires phase velocities to be bracketed by the sub-Moho compressional velocity and that at the base of the lithosphere. In the case of the synthetics, this range is between 8.1654 and 8.2038 km s⁻¹. The resultant rays are quite steep at the receiver, justifying the use of the two-way travel time in equation (1). The $df/2$ offset of the spectral peaks is due to a π -phase shift between multiples¹². The width of the spectral peaks at frequencies given by equation (1) is governed predominantly by the impedance contrast at the boundaries of the layer. Thus the peaks associated with water reverberation, which actually occur between the free surface and the base of the sediments, are quite narrow. The sediment layer, on the other hand, acts as an extremely leaky waveguide for compressional waves because of the low impedance contrast at the ocean-sediment interface. This, coupled with the low value of Q in the sediments, results in broad spectral peaks for sediment reverberations. The power spectrum of a wavelet reverberating

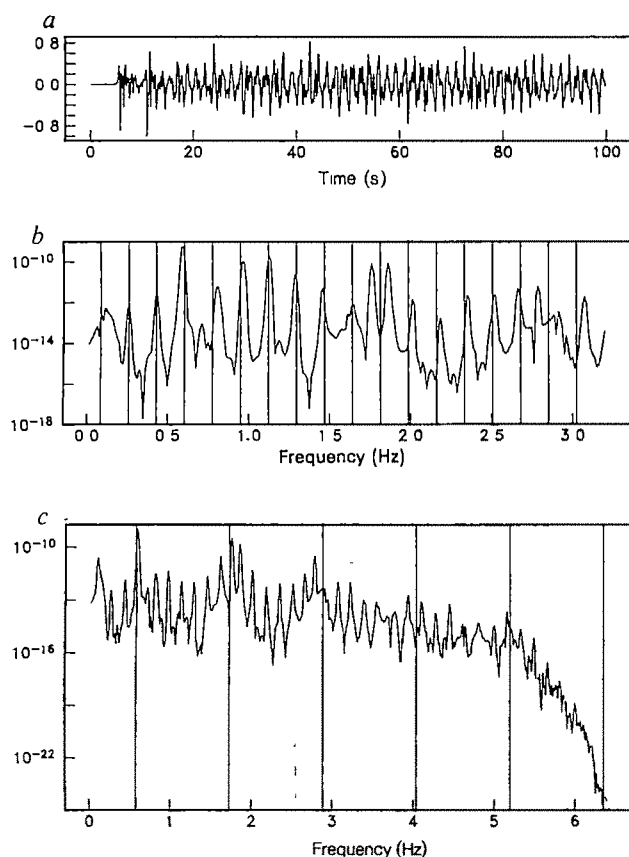


Fig. 2 Power spectrum of the synthetic P_n wavetrain. *a*, Synthetic P_n wavetrain in Fig. 1a. *b*, First 3.0 Hz of the vertical-component power spectrum with vertical lines at frequencies associated with water reverberation. *c*, Horizontal component spectrum with vertical lines indicating predicted positions of spectral humps associated with S-wave sediment reverberation. The synthetics were limited to a Nyquist frequency of 6.4 Hz due to the high computational costs involved.

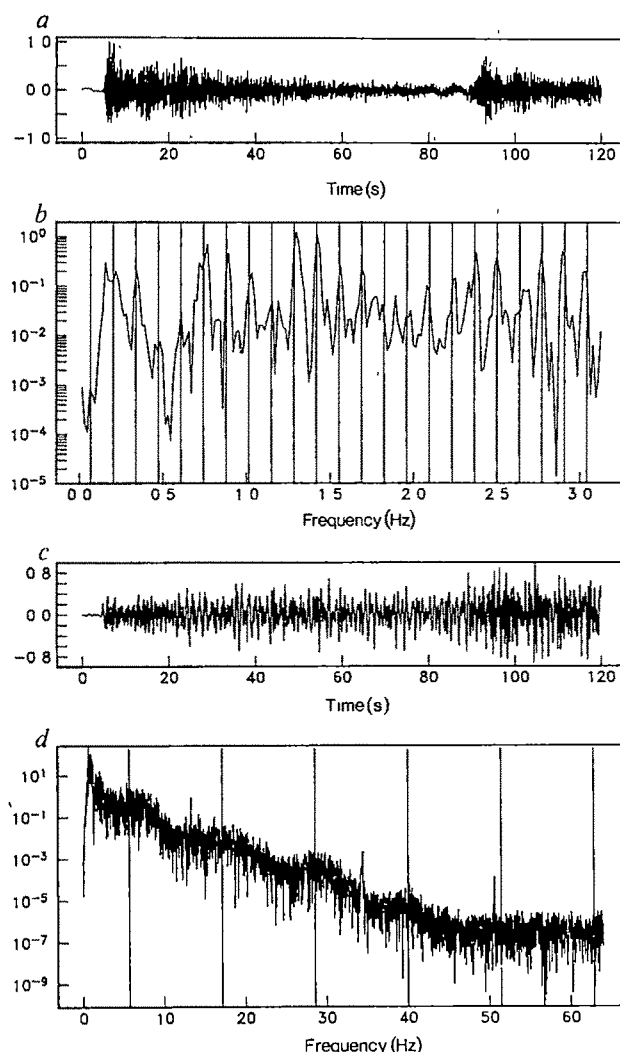


Fig. 3 Data collected in the south-west Pacific during the Ngendei Seismic Experiment. *a*, Hydrophone recording; *b*, the first 3.0 Hz of its corresponding power spectrum with vertical lines at predicted frequencies of water reverberation peaks. *c*, OBS vertical-component seismometer data and *d*, its corresponding power spectrum. Vertical lines indicate predicted positions of spectral humps associated with P-wave sediment reverberation.

in the oceanic water column and sediment layer thus consists of widely spaced spectral 'humps' due to sediment reverberation superimposed on finely spaced spectral 'peaks' associated with water reverberation.

Figure 2 illustrates this behaviour in the synthetic P_n power spectrum. Figure 2a shows the synthetic P_n wavetrain of Fig. 1a. The horizontal component power spectrum in Fig. 2c shows vertical lines indicating predicted positions of spectral humps associated with S-wave sediment reverberation. Figure 2b is the first 3.0 Hz of the vertical-component spectrum with lines at the predicted frequencies of water reverberation peaks. Figure 2 thus clearly illustrates the presence of these reverberations in the synthetic P_n wavetrain.

Data were collected during the 1983 Ngendei Seismic Experiment by an ocean bottom seismometer (OBS) array in the south-west Pacific (23°49' S, 165°32' W), ~900 km east of the Tonga trench¹³. Each OBS contained one vertical and two horizontal seismometers and a hydrophone. Figure 3 shows an example of these data along with their corresponding power spectra.

A clear feature of the P_n phase in Fig. 3c, also seen in the synthetic P_n phase of Fig. 2a, is a predominance of low frequencies late in the wavetrain. Modelling the decay rate of the energy in the signal pictured in Fig. 3c as an exponential of the form $e^{-\gamma t}$ for three separate frequency bands, each two octaves wide,

resulted in values of $\gamma = -0.005$ between 0.5 and 2.0 Hz, $\gamma = 0.003$ between 2.0 and 8.0 Hz and $\gamma = 0.055$ between 8.0 and 32.0 Hz. This result is in direct contrast to models of forward-scattered coda⁶ which predict the fall-off rate to be a decreasing function of frequency. This feature can, however, be explained as the superposition of different modes of reverberation.

The hydrophone is a more narrow band instrument than the OBS vertical component seismometer and is better able to isolate the water reverberations and their corresponding spectral peaks as depicted in Fig. 3b. The vertical-component seismometer, on the other hand, can record the dominant frequency associated with sediment reverberation and clearly exhibits the corresponding spectral humps in Fig. 3d. The predicted frequencies of spectral peaks in both Fig. 3b, d were calculated using values of the sediment and water layer velocities and thicknesses obtained in an independent study¹⁴. When comparing the synthetic spectrum (Fig. 2c) with the data spectrum (Fig. 3d); it is important to realize that the synthetics were computed up to a Nyquist frequency of only 6.4 Hz whereas the data extend to 64 Hz. Thus the water reverberation peaks, which are separated by <0.2 Hz, when plotted on a scale extending to 64 Hz, appear rather incoherent as in Fig. 3d. To illustrate sediment reverberation in the synthetic spectra, we used a thick sediment layer to reduce the peak spacing. In the synthetics, the spacing between sediment reverberation humps is 1 1548 Hz, while in the data it is 11 43 Hz.

The amplitude of the synthetic P_n phase was within a factor of 2 or 3 of the data amplitudes and decayed as $\text{range}^{-1.3}$, slightly faster than pure geometrical spreading. An event located by the National Earthquake Information Service (NEIS) with a published magnitude of 5.8 and moment of 2.6×10^{24} dyn cm was recorded by three of the OBSs at the Ngendei site. The event was a thrust fault source with an azimuth relative to the receiver of 90° at an approximate epicentral range of 920 km. The absolute amplitudes in the first 20 s recorded for this event ranged from 0.76 to 1.44 V. To obtain the maximum amplitude of the synthetic P_n phase, we used a thrust fault source with an azimuth relative to the receiver of 90° at an epicentral range of 900 km. The moment determined by NEIS was used and, after convolving with the instrument response, the resulting maximum amplitude was 0.420 V. Differences of this order can be easily explained in terms of differences in model parameters, fault orientation and focal depth.

The steep incidence of the rays within the oceanic water column and sediment layer implies that only very local changes in bottom topography can affect the propagation of P_n between a particular source and receiver. As long as the OBS is situated in locally smooth topography, the spectral characteristics of P_n mentioned above will be apparent. The main effect of an irregular bottom topography would be to reduce coherence between OBSs at different sites. If the sites are sufficiently separated as to allow changes in either water or sediment thickness and/or velocity, the resulting P_n coda would suffer a severe reduction in spatial coherence.

The oceanic P_n phase can be explained simply as a set of refractions from the lower lithosphere with subsequent water and sediment reverberations. Synthetic P_n wavetrains with durations of the order of 100 s were generated in the absence of scattering. Thus the propagation of the oceanic P_n phase does not require features of the oceanic lithosphere such as low-velocity channels, anomalously high Q values or thin high-velocity lenses, but rather can be explained in terms of simple models with monotonically increasing velocity-depth profiles containing no lateral heterogeneity.

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Stopping of earthquake ruptures at dilational fault jogs

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Palaeoseismic studies over the past several years have indicated that segments of certain major faults tend to rupture at fairly regular intervals in characteristic earthquakes of about the same size¹. This implies the presence of local structural controls which govern the nucleation and stopping of ruptures. Understanding rupture arrest is important, not only because it governs the size of characteristic earthquakes, but also because deceleration of ruptures results in the radiation of high-frequency energy leading to strong ground motion². I show here that rapid opening of linking extensional fracture systems to allow passage of earthquake ruptures through dilational fault jogs in fluid-saturated crusts is opposed by transient suctional forces induced near the rupture tips³. Rupture arrest may then be followed by delayed slip transfer as fluid pressures re-equilibrate by diffusion.

Mapping of rupture traces within major strike-slip systems has shown that they are generally discontinuous on a range of scales^{4,5}. In the San Andreas system, echelon segmentation of the fault trace is common, with the trace stepping abruptly from one side to another of fault zones up to ~ 2 km wide. High-resolution microearthquake studies have demonstrated that large irregularities observed in the surface fault trace often extend throughout the seismogenic zone to depths of 10 km or more⁶⁻⁸. The mechanical implications of echelon fault segmentation have recently been examined in a two-dimensional static stress analysis⁹. Two important cases are considered (Fig. 1); for ease of reference, irrespective of shear sense or faulting mode, they are designated dilational and anti-dilational jogs, depending on the tendency for areal increase or reduction respectively through the step region. For an anti-dilational jog (for example, left step under right-lateral shear), the elastic interaction between echelon fault segments increases both the mean compressive stress between the segments and the frictional resistance at the segment tips, inhibiting slip transfer across the

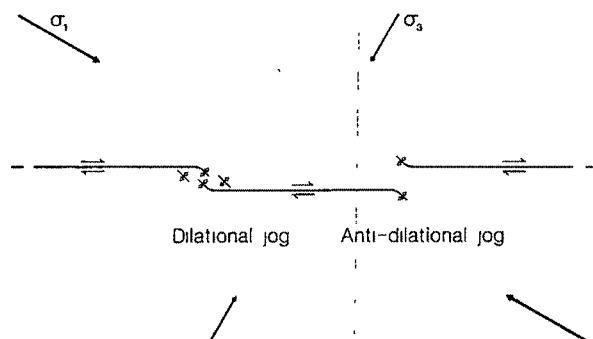


Fig. 1 Likely development of extension fractures in association with dilational and anti-dilational jogs along a segmented fault, shown in relation to far-field principal compressive stresses, $\sigma_1 > \sigma_2 > \sigma_3$ (after ref. 9).

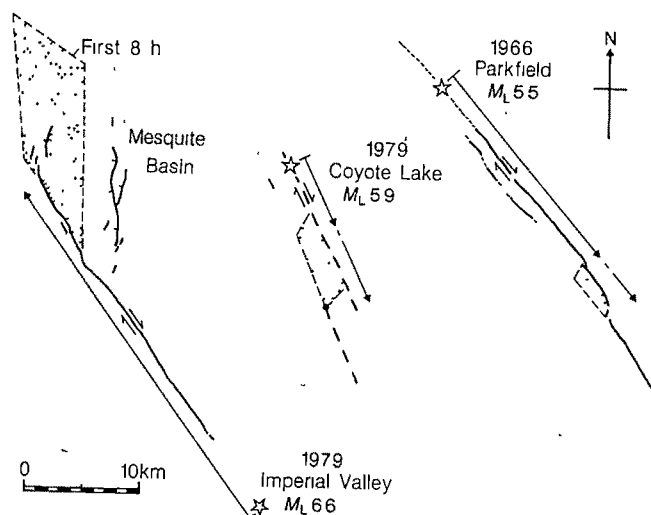


Fig. 2 Seismotectonic sketches illustrating the geometry of strike-slip earthquake ruptures within the San Andreas Fault system (stars, epicentre, arrows, propagation direction and extent of main-shock rupture; broad lines, surface breaks, dash-dot lines, micro-earthquake lineaments, stippling, rhomboidal areas of intense aftershock activity).

discontinuity. Such 'pinned' discontinuities form potential nucleation sites for moderate to large earthquakes^{7,9}. In contrast, for a dilational jog (for example, right step under right-lateral shear), frictional resistance at the segment tips is diminished, facilitating sliding, while mean compressive stress decreases throughout the inter-segment region, favouring the development of a linking fracture system involving extension and/or shear.

Thus, on the basis of this static analysis, echelon fault segmentation involving dilational jogs should present no major hindrance to the transfer of slip along fault systems⁹. Paradoxically, however, several recent strike-slip earthquake ruptures have terminated in the vicinity of dilational jogs which appear to have acted as kinetic barriers opposing rupture propagation. Seismotectonic sketches illustrating the rupture geometry in each case are given in Fig. 2.

The 1966 Parkfield earthquake (of M_L 5.5) is of particular interest as the latest in a series of characteristic $M_L \sim 5.5$ events that have previously ruptured the same Parkfield-Cholame segment of the San Andreas fault in 1934, 1922, 1901, 1881 and also possibly in 1857 as an immediate foreshock to the $M \sim 8$ event which ruptured through the 'Big Bend'^{10,11}. Waveform similarities suggest that the last three events, at least, share essentially the same rupture characteristics, with the implication of strong controls on the nucleation and stopping of these characteristic events. Final ground breakage associated with the 1966 event extended for ~ 35 km (ref. 12). Structurally significant features along the trace include a slight restraining bend (equivalent to an anti-dilational jog) associated with the epicentre, and a pronounced ~ 1 -km dilational jog between echelon segments across the Cholame Valley. While dextral strike-slip at the surface eventually reached ~ 0.2 m near the centre of the rupture, most, if not all of it developed by afterslip over a period of 2 months following the main shock¹³. On the basis of seismic strong motion data and the aftershock distribution, it has been argued that the main co-seismic rupture was vertical, extended from 3 to 8 km depth and propagated unilaterally southeastwards for ~ 25 km from the epicentral bend, with a mean slip of 0.6 m, to terminate in the vicinity of the right-step¹⁴. The surface fault break was discontinuous across the jog, but aftershock activity was particularly intense beneath a 3.5×1 km rhomboidal area immediately north-west of its inferred position, indicating that the echelon segmentation persisted throughout the seismogenic regime to a depth of around 12 km (ref. 6). There are indications of relative subsidence within this rhombic area over a period of weeks following the earthquake¹². The 8-km fault break extending south-east of the jog was probably

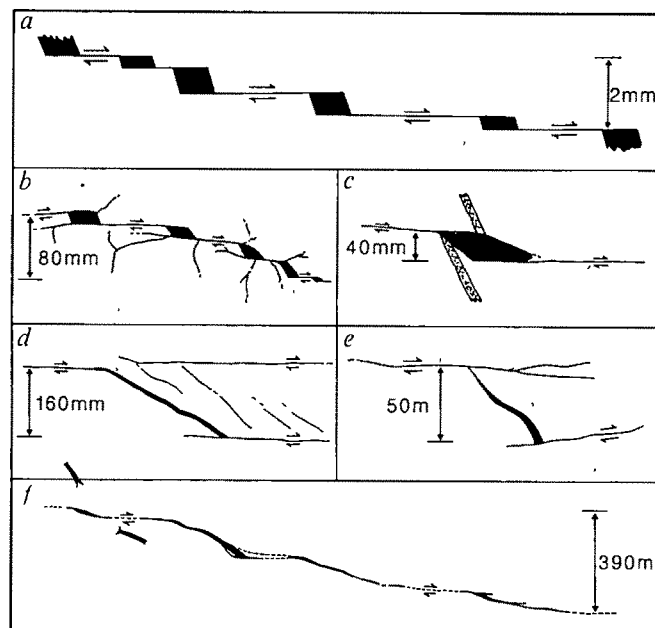


Fig. 3 Dilational jogs in various host rocks on a range of scales from natural fault systems (all drawn for dextral-shear sense). Black areas represent infillings of hydrothermal minerals or cemented breccias (a, quartz-chlorite fibrous infilling in metagreywacke²⁴; b, calcite infilling in flysch²⁵; c, quartz-epidote infilling in granodiorite²⁰; d, quartz infilling in granodiorite⁹; e, gold-quartz vein in greenstones²³; f, multiply quartz cemented breccia-vein in andesitic volcanics²²).

entirely the product of post-mainshock afterslip which decreased logarithmically with time along with the aftershock activity¹³.

Information on the 1979 Coyote Lake earthquake (M_L 5.9) on the Calaveras Fault is based largely on high-precision aftershock studies and waveform analyses, because no clear surface break was observed. However, the seismological data indicate behaviour similar to the Parkfield event, with the main rupture propagating unilaterally southeastwards to terminate adjacent to a dilational jog delineated by intense aftershock activity. Mainshock slip averaging ~ 0.2 m was largely confined to a subvertical rupture extending from 3 to 10 km depth, which propagated southeastwards from the focus for 6–14 km (refs 15, 16). Some 5 h after the main earthquake, beginning with an M_L 4.0 event, aftershock activity extended ~ 2 km south-west to an echelon fault segment and migrated southeastwards over a period of days to define a subsidiary slip plane 8 km in length at depths between 3 and 7 km (ref. 8). Diffuse aftershock activity occurred within the overlap zone.

In the 1979 Imperial Valley earthquake (M_L 6.6), the rupture propagated unilaterally northwestwards for ~ 35 km with an average co-seismic slip of ~ 0.4 m, terminating at a larger-scale dilational jog defined by the down-thrown Mesquite Basin. Surface faulting occurred only on the northern three-quarters of the rupture along the Imperial fault, and also along the subsidiary Brawley fault. Detailed analysis of the main rupture on the Imperial fault¹⁷ shows that the amount of strike-slip, the slip rate and the rupture velocity diminish abruptly north of the intersection with the Brawley fault, but that the rupture continued on past a small restraining bend to stop finally where the strike of the Imperial fault veers northwards. In the first 8 h following the main shock, aftershocks were almost entirely concentrated beneath a 7×11 km rhomboidal area extending north from the main rupture termination¹⁸.

From their geomorphic expression, it seems clear that many of these dilational jogs are long-lived structures with substantial strike-slip motion transferred across them through some form of linking fracture system. Evidence on the nature of these fracture systems comes from several sources. Experimental work on brittle failure has demonstrated the strong tendency for cracks

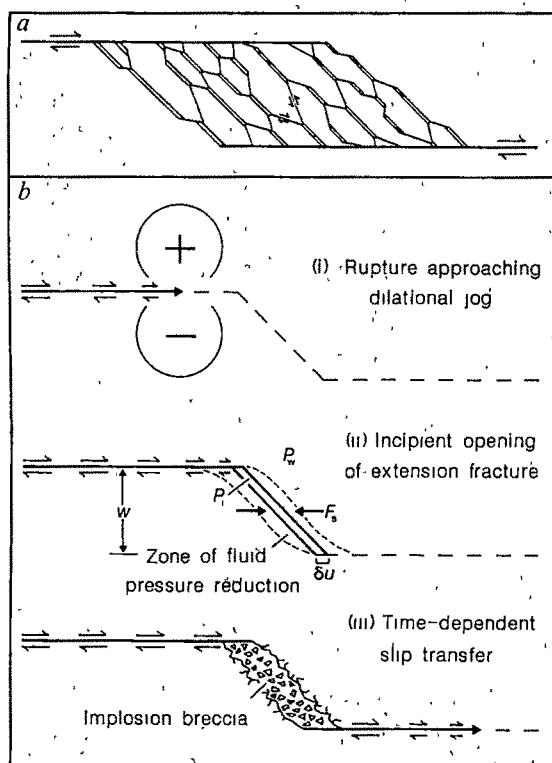


Fig. 4 a, Mixed extensional-shear fracture mesh model linking echelon fault segments across a dilational jog. b, Schematic representation of a dextral rupture with fields of compression (+) and dilatation (-) at the rupture tip encountering a dilational jog consisting of a single linking extension fracture (not to scale).

under shear (fault segments) to propagate into and terminate in; or become linked by extension cracks aligned perpendicular to the least principal compressive stress¹⁹; the same seems to hold true within natural fault systems²⁰. The diffuse, high b -value swarm seismicity sometimes associated with large dilational jogs has been satisfactorily modelled as resulting from a fracture system incorporating short shears linked by vertical extension fractures into a honeycomb mesh²¹ (see Fig. 4a). This is consistent with focal mechanisms obtained from aftershocks within the jogs which show a preponderance of strike-slip solutions with, rarely, some minor normal-slip^{6,8,21}. Smaller examples of dilational jogs, which have been described extensively on a range of scales from ancient fault zones^{9,20,22-25}, are also instructive. Echelon, planar fault segments tend to be linked through the jog regions by individual or multiple extension fractures, occasionally with subsidiary shears, or by the development of more extensive rhombic or lensoidal openings (Fig. 3). The linking fractures are commonly infilled by hydrothermal mineralization, forming extension veins, or contain breccias of wall-rock fragments cemented by hydrothermal minerals^{22,26,27}. Textural evidence often suggests a history of incremental opening for the extension veins, or of multi-episode brecciation and cementation^{22,26,28}. While they are clearly fault-related, the low clast/matrix ratios of these re-cemented breccias indicate significant dilation with included clasts of wall-rock remaining angular and showing little evidence for a derivation by frictional attrition²⁹. The important geometrical conclusion, however, is that the total extensional opening across the linking fracture system in the direction of fault movement is generally comparable to the slip on the echelon fault segments.

Assuming that the internal structure of the larger dilational jogs is analogous to that observed in these smaller-scale examples, we consider the problems associated with transferring co-seismic slip for moderate or larger earthquakes (>0.1 m, say) across such features if, as seems likely on comparative grounds, such slip transfer requires extensional opening of the same order within the jogs. Although the linking fracture system may resemble the mixed extensional-shear mesh model for

earthquake swarms²¹ (Fig. 4a), we represent it simply by an equivalent single extension fracture (Fig. 4b). While the deviatoric stress field within dilational jogs favours the opening of linking extension fractures⁹, static stress analysis does not consider problems associated with the rapid opening of such fractures in fluid-saturated crust. Throughout the seismogenic regime, fluid pressures may reasonably be assumed to be at least hydrostatic²⁸, increasing with depth at ≥ 100 bars km^{-1} . Unless the extensional opening takes place sufficiently slowly for the internal fluid pressures within the linking fractures (P_f) to remain the same as those in the wall-rock (P_w), the opening will be opposed by an induced suctional force (F_s) in the shear direction,

$$F_s = \Delta P w v \quad (1)$$

where ΔP is the average fluid pressure differential, ($P_w - P_f$), v is the vertical extent of the rupture and w is the width of the dilational jog (Fig. 4). Clearly, the magnitude of the induced pressure differential will depend on the porosity and fluid diffusivity of the surrounding rock mass. Fluids could enter the dilating fracture system either by channel flow along the fault segments or from wall-rocks adjacent to the fractures. The former can be neglected given the width of the jogs and the 1-10-s slip durations characteristic of moderate to large ruptures³⁰. At any depth within the seismogenic regime (approximately the top 10 km of crust for geothermal gradients of 25°C km^{-1}), the incompressibility of water is sufficiently high for a fluid volume increase $\leq 10\%$ to lower the fluid pressure to a negligible fraction of initial hydrostatic values³¹. For an infinite planar extension fracture opening an amount, δu , in a time, t , we can define an effective diffusive volume extending a distance, $L = (ht)^{1/2}$, each side of the fracture, where h is the fluid diffusivity of the wall-rock. If the wall-rock has a porosity, p , it follows that the conditions for creation of large fluid pressure differentials is

$$\delta u > 0.2 p L = 0.2 p (ht)^{1/2} \quad (2)$$

Taking a reasonable porosity of 5%, the 1-10-s range of earthquake slip durations, and estimates of fluid diffusivity in fault zones which range from $\sim 10^{-8} \text{ m}^2 \text{ s}^{-1}$ for impermeable clay gouge³² to bulk values³ of $1 \text{ m}^2 \text{ s}^{-1}$, δu must have minimum and maximum values of $1 \mu\text{m}$ and 30 mm , respectively, for large fluid pressure differences to develop. Because we are concerned with transferring slip >0.1 m across the jogs, an induced pressure differential approaching the initial hydrostatic value at any depth therefore seems likely, even if many extension fractures are involved in the linking system. With the tensile strength of rocks rarely exceeding 100 bar, it is easy to see how the high-dilation breccias described above may form by hydraulic implosion of the wall-rocks into extending fractures as a result of these transitory fluid pressure differences (Fig. 4) (see refs 27, 29). For a dilational jog extending to a depth of 10 km, the average pressure differential $\Delta P \approx 500$ bar and from equation (1), F_s scales with the width of the jog for ruptures occupying the entire seismogenic zone. The work done in opening linking extensional fractures against the resistive suctional force is

$$W_s = \delta u F_s = \delta u w v \Delta P \quad (3)$$

For appropriate values, $v = 10 \text{ km}$, $w = 1 \text{ km}$ and $\delta u = 0.1 \text{ m}$, $W_s = 5 \times 10^{13} \text{ J}$. From the Gutenberg-Richter relationship³³, this is comparable to the wave energy released by an earthquake of surface wave magnitude $M_s 6$ (similar to those under discussion), giving some idea of the extent to which the opening of extension fractures within a dilational jog may drain energy from a propagating rupture tip.

The difficulties in transferring earthquake slip across the larger jogs are thus apparent. Rapid opening of extension fractures is strongly opposed by induced fluid pressure differentials, while such drops in fluid pressure as occur within the jog cause dilatancy hardening, increasing effective normal stress and shear resistance on subsidiary shears. The stopping mechanism also provides an explanation for the observed time dependence of

slip transfer and aftershock migration across dilational jogs. Strain-energy concentrations around the end of the arrested rupture may be slowly dissipated by slip transfer across the jog as fluid pressures re-equilibrate by diffusion to allow the necessary extensional opening within the linking fracture system. Similar control of aftershock activity by fluid diffusion has been suggested before³⁴, but not at such specific structural sites. The range of possible fluid diffusivities, noted previously, is sufficiently large for the rate of slip transfer to vary considerably, as is observed. Time-dependent displacement and fluid transfer around the dilational jog at Parkfield should clearly be closely monitored following the next characteristic M_L 5.5 event, which is expected in 1988 ± 4 yr (ref. 10).

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Strontium content of ostracods indicates lacustrine palaeosalinity

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Individual non-marine ostracod valves contain Sr in proportion to the Sr content of their host lake water. Two commonly coexisting, large planktonic species, *Mytilocypris praenuncia* and *Australocypris robusta*, have a Sr distribution coefficient K_D (Sr/Ca) of 0.082 which is independent of temperature in the range 10–18 °C. If the relationship between salinity and the Sr content of a hydrologically simple lake is known, palaeosalinities can be calculated from fossil ostracods. We present here palaeosalinities for the past 10,000 yr for Lake Keilambete, Victoria, calculated from ostracod Sr/Ca of fossil material which are in striking agreement with independent interpretations of palaeosalinity. The range of salinity spans 14–95‰. We also show that a reliable quantitative continuous indicator of lacustrine palaeosalinity is important for the elucidation of continental palaeoenvironments for palaeoclimatology and archaeology and ocean-continent climatic correlations and interactions.

The rapid advance in the knowledge of Quaternary global climate, stemming largely from the study of Foraminifera from

the ocean basins¹⁻³, has not been matched by equivalent progress in continental environments. Lacustrine sediments can supply information on changing continental climate. Those techniques commonly applied to lacustrine sediments, including sedimentary facies analysis, palynology, documentation of biota (such as ostracods and diatoms) with diagnostic salinity or temperature tolerances produce useful limiting ranges of palaeoenvironmental conditions. Oxygen-isotope studies of authigenic or biogenic lacustrine precipitates are commonly hampered by unknown previous $\delta^{18}\text{O}$ water compositions, which may have varied as a function of salinity (precipitation/evaporation ratio), precipitation regime and temperature and varying contributions from multiple fluvial and/or groundwater sources. What is lacking is a method capable of eliciting continuous salinity or temperature variations from lacustrine sediment cores.

Ostracods are considered to be ideal monitors of water quality because, before reaching maturity, they rapidly shed their shells several times (up to nine times, at approximately weekly intervals) during growth and the source material for their shells comes entirely from the host water⁴. We have shown previously⁵ that the Mg content of some non-marine ostracod shells is a function of salinity and temperature and that coupled Mg/Ca and $\delta^{18}\text{O}$ measurements might provide a record of combined palaeotemperature and palaeosalinity. However, the Sr/Ca ratios of the calcitic carapaces of the ostracod *Mytilocypris henricae*, grown in controlled conditions in aquaria and from natural lakes⁶, appear essentially independent of temperature (T) in the range 10.5–25 °C. Specimens of *A. robusta*, which is a species phylogenetically related to and of similar shape and size to *M. henricae*, collected at different seasons ($T = 10.2$ –18 °C) from Lake Keilambete in western Victoria (lat. 38°12' S; long. 142°53' E), share the same chemical relationship (Table 1). This raises the possibility that the Sr content of some non-marine ostracods may be used to determine palaeosalinity through the former Sr contents of the water.

A reconnaissance study of Sr/Ca ratios of carapaces from live ostracods from a series of south-east Australian lake waters⁵ of salinity 1.3–73‰ suggested that the Sr content of the shell was a direct and positive function of the Sr content of the lake water. We examined the Sr/Ca ratios of shells of 18 species, namely, *Australocypris* (two species), *Mytilocypris* (five species), *Diacypis* (three species), *Reticypis*, *Cyprinotus*, *Limnocythere* (three species), *Ilyocypris*, *Cypricercus* and *Cypretta*. We conclude that the distribution coefficient, K_D (Sr/Ca) = $[\text{Sr/Ca}]_{\text{CaCO}_3}/[\text{Sr/Ca}]_{\text{H}_2\text{O}}$, may be species dependent, although to a smaller degree than is the K_D (Mg/Ca) (ref. 5). The larger genera *Mytilocypris* and *Australocypris* have identical K_D (Sr/Ca). For modern *A. robusta* from Lake Keilambete (Table 1), K_D (Sr/Ca) ~ 0.082 .

For the first application of the Sr/Ca palaeosalinometer, we examined fossil ostracods from a lake which has simple hydrological characteristics. Lake Keilambete is a near-circular maar lake^{7,8} (area of lake 2.7 km²; area of catchment, that is, to crater rim = 4.2 km²) with input received only by direct precipitation, and water loss only by evaporation⁷. Thus, lake levels and the concentration of dissolved salts reflect changes in precipitation/evaporation, and documentation of sedimentary facies variations⁸ and palynology⁹, and identification of diagnostic ostracod species¹⁰ from cores spanning the past 10,000 yr have been used previously to infer changes in climate. The chronology of core KN (Fig. 1), representing 10,000 yr of sedimentation in 415 cm, is well controlled by 18 ¹⁴C dates^{7,9,11} that have been correlated from adjacent cores by comparison with a distinctive sequence of white aragonite and calcite microlaminar.

The Sr/Ca analyses of individual ostracods are presented in Fig. 1a. The $[\text{Sr/Ca}]_{\text{CaCO}_3}$ has been expressed as palaeosalinity using K_D (Sr/Ca) ~ 0.082 and the 1983 lake composition (mean salinity 73‰, and mean $(\text{Sr/Ca})_{\text{CaCO}_3}$ of 0.00635). This assumes that values for both salinity and Sr content are conservative and that the Ca content of the water is unimportant. *M. praenuncia* is generally present from 0 to 290 cm; ostracods are scarce in the interval 290–350 cm; and from 350 to 415 cm *A. robusta* is

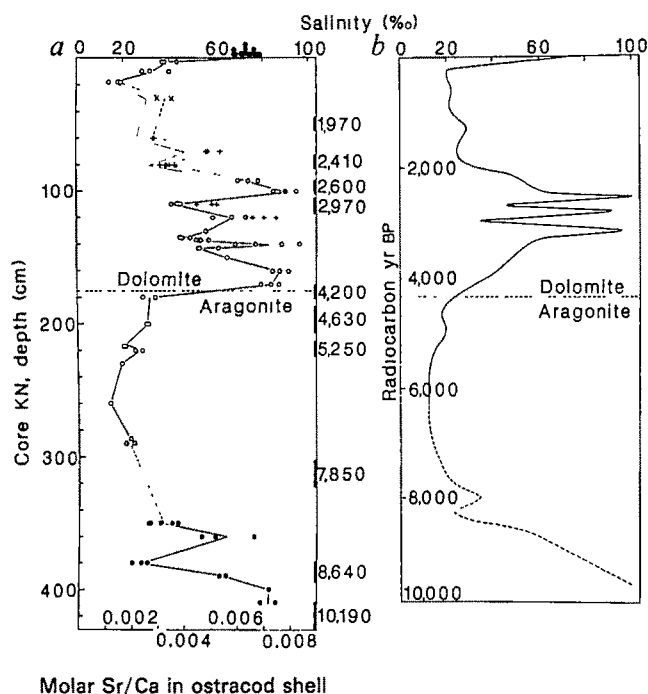


Fig. 1 *a*, Palaeosalinity curve for Lake Keilambete based on Sr/Ca composition (lower scale) for ostracods from 34 horizons within core KN. The data are principally from *Australocypris robusta* (●), *Mytilocypris praenuncia* (○), with some results for *Leptocythere lacustris* (×) and *Diacypris compacta* (+). Sr/Ca results for coexisting *M. praenuncia* and *D. compacta* at 110 and 120 cm suggest that *D. compacta* may incorporate more Sr ($K_D(\text{Sr/Ca}) \sim 0.10$) at the same conditions than does *M. praenuncia*. Between 20 and 90 cm, the dashed line records the Sr/Ca variations for *D. compacta* and *L. lacustris*. The parallel dotted line at lower salinity based on the species differences in $K_D(\text{Sr/Ca})$ may be a better indicator of salinity. Seventeen Sr/Ca values for modern *A. robusta* are incorporated in a histogram above zero depth. The horizontal dashed line at 175 cm indicates the first appearance of authigenic dolomite. *b*, Palaeosalinity curve (smoothed, based on 40 samples) derived from sediment grain-size analysis⁸. A calibration of the proportion of the >44-μm fraction of modern bottom muds as a function of present water depths (from 0 to 11 m) was applied to the proportion of the >44-μm fraction of samples from core K4.

present. At one horizon (100 cm) both species are recorded, and each has a similar Sr/Ca value. Analysis of up to five specimens from some levels show a tight cluster of Sr/Ca results. Between 20 and 90 cm, there is a paucity of *M. praenuncia* and we show the Sr/Ca results for *Leptocythere lacustris* and *Diacypris compacta*. We have insufficient information from modern lakes to establish $K_D(\text{Sr/Ca})$ for these species, but their analyses appear to fit within the trend described by analyses of the two principal species. The trend also compares favourably with Bowler's⁸ palaeosalinity curve interpreted from sedimentary grain-size analysis (Fig. 1*b*). We suggest that the $K_D(\text{Sr/Ca})$ for all these species may be similar.

There are several striking similarities between the palaeosalinity curves derived from $(\text{Sr/Ca})_{\text{ostracod}}$ and by grain-size analysis. The most saline water (at 2,600 yr BP, 100 cm) is estimated to have been ~100% by grain-size analysis and 90–95% by Sr/Ca. Similarly, the lowest salinity at 6,400 yr (260 cm) is estimated to have been 12 and 14% respectively. Both methods assume constancy of salts in the lake. One is based on fossil chemistry indicating past water chemistry, the other on texture of sediments to indicate past water levels, which in turn indicate palaeosalinity in a closed system. The close correspondence between the two independent sets of results is strong evidence for the correctness of the assumption. The significant difference in results from the two methods is that the Sr/Ca data suggest that a period of high salinity began earlier (at 4,200 yr, 175 cm) than that estimated by grain-size analysis. The interpretation based on Sr/Ca is logical, because at 175 cm and higher in the core, authigenic dolomite displaces authigenic aragonite. An abrupt increase in salinity (Mg content) at this point is likely. The previously documented salinity tolerances^{10,12} for *M. praenuncia* and *A. robusta* are 5–43% and 7–145%, respectively. Our data suggest that *M. praenuncia* existed at up to 95% salinity.

Further comment on the palaeoclimatic implications of the Lake Keilambete region would only repeat Bowler's work^{7,8}. We conclude that the Sr/Ca of non-marine ostracods faithfully records palaeosalinity. Those species that have large salinity tolerances (for example 3–150%) are exceedingly useful, and their Sr/Ca provides a potentially continuous palaeosalinometer, free of the discontinuities inherent in the estimation of palaeosalinity by recognition of biota with limited and specific salinity ranges. The reliability of this method will be governed by the hydrological setting of those lakes studied. Small tectonically stable closed basins seem suitable; volcanic and impact crater basins which have negligible groundwater

Table 1 Partial chemical composition of lake water and individual calcitic valves of the ostracod *Australocypris robusta* from Lake Keilambete at three different seasons

	Lake water				Individual ostracod valves				Distribution coefficient
	<i>T</i> (°C)	<i>S</i> (‰)	<i>pH</i>	<i>Ca</i> ²⁺ (p.p.m.)	<i>Sr</i> ²⁺ (p.p.m.)	Molar Sr/Ca	<i>Ca</i> (μg)	<i>Sr</i> (μg)	<i>K_D</i> (Sr/Ca)
11 October 1982 (spring)	13.5	74	8.2	21.1	3.6	0.0780	33.64 39.73 41.13 44.05 53.38	0.4897 0.5912 0.5053 0.6094 0.7371	0.00666 0.00681 0.00562 0.00633 0.00632
									Mean 0.0814 ± 0.006 (1σ)
6 August 1983 (winter)	10.2	71.5	9.0	21.2	3.6	0.0777	2.87 44.57 52.52 56.72 59.24	0.0394 0.6180 0.6813 0.7708 0.7690	0.00628 0.00634 0.00593 0.00622 0.00594
									Mean 0.0790 ± 0.003 (1σ)
8 December 1983 (summer)	18.0	72.7	8.9	21.4	3.5	0.0748	16.74 22.21 34.50 39.94 41.06 45.98 55.19	0.2440 0.2866 0.4662 0.5620 0.6159 0.6216 0.7908	0.00667 0.00590 0.00618 0.00644 0.00686 0.00618 0.00655
									Mean 0.0855 ± 0.004 (1σ)

Salinity (*S*‰) equated to the total dissolved solutes by the formula of Williams¹⁴. Distribution coefficient, $K_D = [\text{Sr/Ca}]_{\text{CaCO}_3} / [\text{Sr/Ca}]_{\text{H}_2\text{O}}$. Chemical analyses^{5,6} were by very high-resolution inductively coupled argon plasma emission spectrometer (ICPAES). All concentrations were greater than an order of magnitude above the detection limits which are Ca^{2+} 0.02 p.p.b. (parts per 10⁹) and Sr^{2+} 0.03 p.p.b. in solution.

inflow or leakage are ideal. Because the varying $\delta^{18}\text{O}$ value of water during evaporation can be largely predicted as a function of salinity¹³, once palaeosalinity is determined from ostracod Sr/Ca, a semi-continuous record of calculated $\delta^{18}\text{O}$ of lake water might be coupled with ostracod Mg/Ca (ref. 5) or $\delta^{18}\text{O}$ of ostracod valves or other calcareous biota to indicate lacustrine palaeotemperatures.

At present, the interpretation of continental palaeoclimatic changes is largely restricted to consideration of changes in precipitation/evaporation (P/E), without indication of whether these variations are due to changes in precipitation (P), evaporation (E), or both. A resolution of quantitative continental palaeotemperature and lacustrine palaeosalinity would allow an independent estimation of past precipitation and temperature regimes.

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Adaptation of photosynthetic apparatus of marine ultraphytoplankton to natural light fields

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The discovery of widely distributed populations of chroococcoid marine cyanobacteria (*Synechococcus* spp.) deep in the water column has focused attention on the autotrophic potential of small (<10 μm) size classes of marine phytoplankton^{1–5}. These organisms contain light-harvesting pigments which specifically absorb in the blue and green regions of the spectrum^{6–11}. Despite the fact that sea water acts as a monochromatic filter, allowing only the blue and blue-green wavelengths to penetrate¹², essentially all estimates of the photosynthetic rate of ultraphytoplankton at depth have ignored the changing spectral composition of the natural light field and simulated only the *in situ* light intensity^{4,5,13}. Here I show that the spectral composition of available light must be considered in estimates of water column productivity and that the depth of the euphotic zone varies between different ultraphytoplankton strains depending on the composition and organization of the photosynthetic apparatus.

Four experiments were performed, each modelled on the work of Jenkin¹⁴. In experiments 1–3, the photosynthetic performance of two phycoerythrin (PE)-containing *Synechococcus* clones was compared with that of PE-lacking *Synechococcus* clones at different light intensities in artificial white light (AWL) and at 15, 30,

45 and 60 m depths in the euphotic zone of clear ocean water (Table 1). In almost all cases, the cyanobacteria showed higher rates of photosynthesis in AWL than in natural light of comparable intensity, indicating that simulated *in situ* techniques probably overestimate the contribution of *Synechococcus* to primary productivity at depth. In AWL, the effect of decreasing light intensity was approximately equal for all the clones; the cellular rate of photosynthesis at 37 $\mu\text{E m}^{-2} \text{s}^{-1}$ was $\sim 20\%$ ($\pm 3\%$) of the rate at 260 $\mu\text{E m}^{-2} \text{s}^{-1}$. In contrast, the photosynthesis-irradiance relationships in natural light show marked differences between clones. In experiment 3, clone WH5701 showed no detectable ¹⁴C uptake at 60 m, although the two PE-containing clones, WH8018 and WH7803, showed photosynthesis rates of 6.78 and 8.42 fg C per cell per h, respectively. Thus, while the PE-lacking clone had reached its compensation light intensity, the rate of photosynthesis of clone WH8018 was decreased by only 70% compared with its rate at 15 m; the photosynthetic rate of clone WH7803 at 60 m was similarly decreased by only 67%. This pattern was repeated in all three experiments with the cyanobacteria; the photosynthetic rate at 60 m compared with that at 15 m was inhibited to the greatest extent in the PE-lacking clones, to a lesser extent in clone WH8018, and to the least extent in clone WH7803.

This rank order corresponds to the presence of accessory light-harvesting pigments which absorb light at wavelengths that penetrate to 60 m. The PE-lacking clones contain phycocyanin (PC) and allophycocyanin (APC) as their principal accessory pigments; these pigment proteins have absorption maxima at 620 and 650 nm, respectively¹⁵. Clone WH8018 contains PC and APC, but also contains a PE closely resembling C-phycoerythrin as the major light-harvesting pigment; this PE contains only phycoerythrobilin (PEB) chromophores (absorption maximum = 550 nm)^{7,16}. WH7803 also contains a PE as its major light-harvesting pigment; this PE, however, contains both PEB and phycobilin (PUB) chromophores. The PUB chromophore has an absorption maximum at 492 nm and its presence enhances light-harvesting ability at blue wavelengths¹⁶.

The wavelength (λ) of maximum absorption *in vivo* and the presence or absence of different phycobiliproteins in marine *Synechococcus* spp. are under genetic control and independent of cell nutrient status¹⁷. Thus, the rank order for *in situ* photosynthesis observed in the present study should also be observed in natural populations. The relative differences between clones are probably not as great as would be seen in nature, as the cultured cells were grown in the presence of excess nutrients and undoubtedly contain more total pigment per cell than would individuals growing *in situ*. Increased total absorbance associated with high cellular pigment concentration would partially compensate for poorer relative absorption of blue light by genotypes which lack PE or contain a PE composed of only PEB chromophores.

Phycobiliproteins are closely related and are thought to have arisen by duplication of a common ancestral gene sequence^{18–22}. On the basis of subunit structure^{9,21}, sequence homology^{18–22}, distribution among taxa²², serological affinity²² and *in vivo* organization^{15,23,24}, the phycocyanins and allophycocyanins are viewed, phylogenetically, as the older forms^{18–22}. Phycoerythrins, particularly those containing both PEB and PUB chromophores, evolved more recently^{18–22}. In the marine *Synechococcus*, it seems that natural selection has favoured this evolutionary progression since, as shown here, each molecular step which increases the complexity of the photosynthetic apparatus, also increases the depth at which net photosynthesis can be achieved.

The theory that photosynthetic marine organisms possess pigments which complement the spectral composition of available light is an old one^{25,26} which has been widely accepted despite contrary empirical evidence for a number of species^{27,28}. The data presented here support the theory and suggest that complementary chromatic adaptation is an evolutionary rather than a physiological process in marine *Synechococcus* spp. If this is the case, the PE-containing genotypes, particularly those

Table 1 Comparison of photosynthetic rates of different clones

Experiment	Environmental conditions	Total PAR ($\mu\text{E m}^{-2} \text{s}^{-1}$)	% Surface irradiance	Rate of photosynthesis (fg C per cell per h)		
1	AWL	260	41.6	WH7803	WH8018	WH8007
	AWL	70	11.2	90.6	57.3	179.8
	AWL	36	5.8	41.8	23.1	84.4
	15 m	180	28.8	16.8	9.6	35.6
	30 m	90	14.4	60.5	35.1	41.3
	45 m	53	8.5	52.1	31.5	23.4
	60 m	33	5.3	39.9	12.6	18.7
2	AWL	260	33.3	WH7803	WH8018	WH8007
	AWL	70	9.0	85.7	88.4	114.4
	AWL	36	4.6	42.3	44.7	50.9
	15 m	220	28.2	15.7	22.3	23.8
	30 m	122	15.6	40.2	55.6	46.8
	45 m	70	9.0	36.7	56.3	—
	60 m	38	4.9	43.5	38.3	19.6
3	AWL	260	26.9	WH7803	WH8018	WH5701
	AWL	70	7.3	47.3	62.2	24.5
	AWL	36	3.7	—	28.7	24.9
	15 m	260	26.9	8.1	14.9	5.0
	30 m	115	11.9	20.4	27.7	7.3
	45 m	55	5.7	19.5	24.4	5.2
	60 m	28	2.9	24.5	16.9	5.4
4	AWL	260	43.3	WH7803	13-1	NEP
	AWL	70	11.7	29.6	3,451.3	1,181.9
	AWL	36	6.0	14.4	2,115.3	576.2
	20 m	240	40.0	5.0	725.4	190.8
	40 m	100	16.7	21.4	3,688.5	910.7
	60 m	40	6.7	10.5	2,242.7	561.9
				4.3	1,049.6	323.0

Values are means of duplicate samples; ND indicates no detectable ^{14}C incorporation; dashes indicate lost samples; AWL, artificial white light. Clonal isolates of the eukaryotic ultraphytoplankton *Thalassiosira oceanica* Hasle and Heimdal (clone 13-1) and *Pavlova* sp. (clone NEP), and marine *Synechococcus* spp. clones WH7803 and WH8018 (which contain PE), and WH5701 and WH8018 (which lack PE) were obtained from the Center for Culture of Marine Phytoplankton (Bigelow Laboratory for Ocean Sciences). Clones 13-1 and NEP were isolated by R. R. L. Guillard in 1958 from surface waters in the Sargasso Sea and Gulf Stream, respectively. Clone WH8007 was isolated in 1980 from surface waters on the continental shelf of the northeastern United States by L. Provasoli. Isolation data for the remaining clones are given elsewhere⁷. The average diameter of the *Synechococcus* clones is 1–2 μm ; that of clone NEP is 6 μm ; and that of clone 13-1 is 3 μm . All experimental work was carried out onboard the R/V *Cape Hatteras* between 29 September and 14 October 1983, at four stations in the eastern edge of the Gulf Stream, between 29° and 34° N. Expendable bathythermograph data showed that the upper 75 m of the Gulf Stream in this region was isothermal at between 27.6 and 28.1 °C. Cultures were maintained in dilute cell suspensions (absorbance, $A_{\lambda\text{max}} \approx 0.3\text{--}0.4$) at the early exponential phase of growth by repeated transfer into f/2-silica or f/2 media³⁶, and incubated at *in situ* temperatures (28 °C) with continuous illumination under Cool White fluorescent lights (General Electric) at an intensity of 50 $\mu\text{E m}^{-2} \text{s}^{-1}$. The cultures had been pre-acclimated to this light regime for 1 month (>10 generations) before the cruise. Before each experiment, the cultures were diluted into fresh sterile media as necessary to achieve approximately equal absorbance values ($A_{\lambda\text{max}} \approx 0.2$), subsampled for cell counts, and spiked with ^{14}C -labelled sodium bicarbonate. The cultures were then subdivided into clear, 50-ml polycarbonate centrifuge tubes (Nalgene), sealed with a cap which allowed compensation for changes in external pressure, and incubated either onboard ship at 28 °C under Cool White fluorescent lights or at different depths in the water column in holders suspended from the ship's hydrowire. The holders were of clear Plexiglass, with clips holding the tubes to a bottom panel. Water circulated freely above and around the tubes. In the shipboard incubator, light was attenuated by neutral-density white Plexiglass panels. At the end of the incubation period, the rate of ^{14}C incorporation into organic material was determined by acidification and bubbling⁷; rates were corrected for dark uptake. Each experiment began approximately 1 h before local apparent noon, and continued for no more than 3 h to minimize the effect of diurnal changes in the spectral composition of solar radiation incident on the sea surface. Skies were partly cloudy during expt 1, and for the first half hour of expt 4, but were clear during expts 2 and 3 and during the last 2½ h of expt 4. The intensity of photosynthetically active radiation (PAR) and attenuation of light at 440, 520, 550 and 670 nm were determined using a Li-Cor underwater quantum meter and a four-channel underwater photometer. At all stations, the optical properties of the water corresponded to Jerlov type I oceanic water¹², with the 1% light level between 90 and 110 m; per cent transmittance in the top 10 m for light at 440, 520, 550 and 670 nm averaged 82.5, 79.8, 63.1 and 3.3%, respectively.

containing a phycoerythrin composed of PEB and PUB chromophores, would be expected to dominate natural populations of *Synechococcus* in oligotrophic waters where the photic zone can exceed 100 m; this appears to be the case as PE-lacking strains have never been found among oceanic isolates (J. B. Waterbury, personal communication) and strains containing a phycoerythrin with PEB and PUB chromophores are much more common among oceanic isolates than strains containing a phycoerythrin with only PEB chromophores^{6,7}. Note also that there is evidence for parallel evolution in *Oscillatoria erythraea* (Trichodesmium), the only other abundant planktonic cyanobacterium in oligotrophic ocean waters; this species also has a PE composed of both PEB and PUB chromophores²⁹.

In experiment 4, the performance of *Synechococcus* clone WH7803 was compared with that of two eukaryotic species: *Thalassiosira oceanica* Hasle and Heimdal (clone 13-1) and *Pavlova* sp. (clone NEP). The results (Table 1) show that while *Synechococcus* spp. containing a phycoerythrin with both PEB and PUB chromophores may have a competitive advantage in deep water compared with other cyanobacteria, the eukaryotes have a greater ability to adapt to the changing spectral composition of light fields in the open ocean. All three clones showed an approximately equal photosynthesis-irradiance relationship in AWL (Table 1), but when the rate of cellular photosynthesis at 60 m was compared with that at 15 m, the *Synechococcus* clone showed the greatest inhibition (Table 1); this is because the

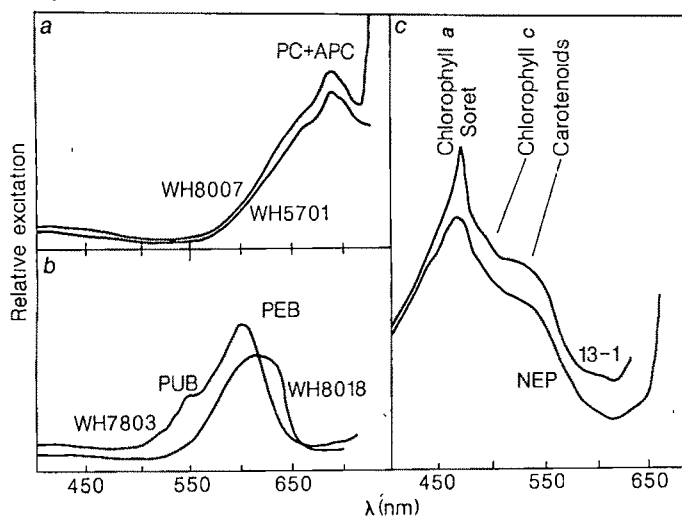


Fig. 1 *In vivo* excitation spectra for fluorescence emission by chlorophyll *a* at 680 nm in the marine *Synechococcus* (a, b) and eukaryotic ultraphytoplankton (c) clones used in the present study. Light energy absorbed by the Soret band of chlorophyll *a* is clearly transferred through the photosynthetic apparatus to a greater extent in the eukaryotes than in the cyanobacteria.

Methods. Cells from exponentially growing cultures were suspended on Whatman GF/B glass fibre filters and spectra obtained in ratio mode on a Baird Atomic SF-1 spectrofluorometer, with excitation and emission bandpass settings of 2 and 32 nm, respectively. Cells had been maintained at 24 °C under continuous illumination (Cool White fluorescent lights) at 50 $\mu\text{E m}^{-2} \text{s}^{-1}$.

Soret band of chlorophyll *a* can drive photosynthesis to a greater extent in the eukaryotes than the marine *Synechococcus* (Fig. 1). This significant difference in the organization of the photosynthetic apparatus of prokaryotic and eukaryotic ultraphytoplankton has not previously been reported and suggests strongly that eukaryotic ultraphytoplankton have the potential to out-compete marine *Synechococcus* at extreme depths where only blue and violet light penetrate, a conclusion which is independently supported by the work of H. W. Glover, M. Keller and R. R. L. Guillard (unpublished). In view of recent reports that small eukaryotic cells are occasionally more abundant than *Synechococcus* at depth³⁰⁻³⁵, eukaryotic ultraphytoplankton must be viewed as a potentially important source of new particulate material in deep ocean water.

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Odorant-sensitive adenylate cyclase may mediate olfactory reception

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The mechanism of the sense of smell has long been a subject for theory and speculation¹⁻⁵. More recently, the notion of odorant recognition by stereospecific protein receptors has gained wide acceptance⁶⁻¹¹, but the receptor molecules remained elusive⁹⁻¹⁵. The recognition molecules are believed to be quite diverse^{9,11,13-15}, which would partly explain the unusual difficulties encountered in their isolation by conventional ligand-binding techniques^{12,13}. An alternative approach would be to probe the receptors through transducing components that may be common to all receptor types^{7-9,12-14}. Here we report the identification of one such transducing molecular component. This is an odorant-sensitive adenylate cyclase, present in very large concentrations in isolated dendritic membranes of olfactory sensory neurones. Odorant activation of the enzyme is ligand and tissue specific, and occurs only in the presence of GTP, suggesting the involvement of receptor(s) coupled to a guanine nucleotide binding protein (G-protein)¹⁵⁻¹⁹. The olfactory G-protein is independently identified by labelling with bacterial toxins, and found to be similar to stimulatory G-proteins in other systems¹⁷⁻¹⁹. Our results suggest a role for cyclic nucleotides in olfactory transduction^{13,20-22}, and point to a molecular analogy between olfaction and visual^{15,16}, hormone^{17,18} and neurotransmitter¹⁹ reception. Most importantly, the present findings reveal new ways to identify and isolate olfactory receptor proteins.

We have recently described a preparation of sensory cilia from frog olfactory neuroepithelium¹¹. These isolated extensions of the chemosensory dendritic membranes are believed to contain the olfactory receptive apparatus¹⁰⁻¹⁴, in much the same way as isolated retinal rod outer segments contain the molecular photoreceptive machinery^{15,16}. We found that the olfactory cilia preparation contains adenylate cyclase at nearly 15 times higher specific activity than membranes from brain or whole olfactory epithelium, both known as rich sources of the enzyme^{20,23} (Fig. 1a). From the change of specific activity after deciliation (Fig. 1a), we calculated that the cilia contain ~40% of the total epithelial adenylate cyclase, although they contain only ~3% of the total protein. Non-sensory cilia prepared from respiratory epithelium showed only 1% of the specific activity found in the olfactory organelles (Fig. 1a). GTP and its non-hydrolysable analogues guanosin 5'-(β , γ -imido)triphosphate (GppNHp) and guanosin 5'-O-(3-thio)triphosphate (GTP γ S), as well as fluoride ions and forskolin, enhanced the olfactory cilia enzymatic activity, while guanosin 5'-O-(2-thio)diphosphate (GDP β S), a GDP analogue, was inhibitory (Fig. 1b). Thus, the basic properties of olfactory adenylate cyclase are similar to those of hormone- and neurotransmitter-stimulated enzymes in other tissues¹⁷⁻¹⁹.

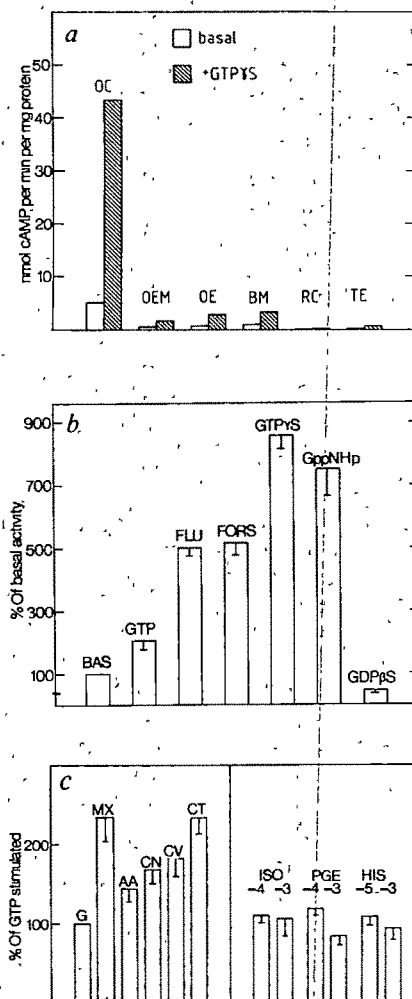
An important criterion for a functional role of adenylate

Fig. 1 *a*, Adenylate cyclase activity of frog olfactory cilia compared with that of membranes derived from other frog tissues. Open bars, basal activity levels; hatched bars, activity stimulated by 10 μ M GTP γ S (10 μ M GppNHp for turkey erythrocytes). OC, olfactory cilia preparation; OEM, membranes from deiliated olfactory epithelium; OE, membranes from whole olfactory epithelium; BM, membranes from brain; RC, respiratory cilia preparation; TE, turkey erythrocyte membranes. *b*, Activation of olfactory cilia adenylate cyclase by various effectors: BAS, basal activity; FORS, forskolin (10 μ M); GTP (10 μ M); FLU, sodium fluoride (10 mM); GppNHp (10 μ M); GTP γ S (10 μ M); GDP β S (1 μ M). *c*, Effect of odorants and other compounds on olfactory cilia adenylate cyclase in the presence of 10 μ M GTP. The results are normalized with respect to activity elicited by GTP alone (G), which is about twice the basal activity level; MX, equimolar mixture of the four indicated odorants (Aldrich) at 0.25 mM each: AA, *n*-amyl acetate; CN, 1,8-cineole; CV, L-carvone; CT, citral (a mixture of *cis* and *trans* isomers); each at 1 mM. ISO, isoprenaline (isoproterenol); PGE, prostaglandin-E₁; HIS, histamine, at log molar concentration as indicated. The lower concentration is in the appropriate physiological range, the higher is for comparison with the maximal odorant concentration used. Error bars in *b* and *c* (as in all following figures) represent standard deviation. Standard deviations in *a* are $\pm 15\%$ in any given preparation and up to $\pm 30\%$ between preparations. However, this larger error in absolute specific activities does not affect the activity ratios expressed in *b* and *c*.

Methods. Frogs (*Rana ridibunda*) were used for cilia preparation as described elsewhere^{10,11}. Briefly, epithelia were dissected and exposed to a 'calcium shock' by abruptly increasing Ca^{2+} from $<1 \mu\text{M}$ to 10 mM. Two steps of differential centrifugation yielded a preparation containing $>80\%$ cilia fragments and membrane vesicles derived from them. Membranes from various frog tissues were prepared by a simplified procedure in which tissue was polytron-homogenized (30 s, 0°C) in 20 mM Tris-HCl, pH 8.0, saturated with phenylmethylsulphonyl fluoride (PMSF). After removal of tissue and intact cells by centrifugation at 500g and 1,700g, respectively, membranes were precipitated at 20,000g. Turkey erythrocyte membranes were prepared as described previously³². Cilia and membranes were stored at -70°C or -180°C and aliquots frozen and thawed only once to keep adenylate cyclase activity intact. Adenylate cyclase was assayed as described previously²⁷ in the presence of 0.5 mM 3-isobutyl-1-methyl xanthine (IBMX). Protein content of samples was measured by the method of Bradford³⁸, with bovine serum albumin as a standard.

cyclase activity in olfaction would be its modulation by odorous compounds in a cell-free system²⁴. To maximize the probability of observing an effect, experiments were carried out with a mixture of four odorants (citral, L-carvone, 1,8-cineol and *n*-amyl acetate), all shown electrophysiologically to be detected by the frog olfactory system^{7,8,13,25-27}. This odorant mixture enhanced the adenylate cyclase activity of olfactory cilia in a dose-dependent manner (Fig. 2a). Activation was already significant at 2.5 μM of individual odorants. In several independent experiments, the maximal activation achieved at 0.25 mM of individual odorants was to 4.6 ± 0.8 of the basal value (2.3 ± 0.4 of the GTP-stimulated level). Odorant stimulation was seen only in the presence of GTP, suggesting dynamic regulation requiring GTP hydrolysis, while GTP γ S stimulated the enzyme maximally, eliminating the odorant effect (Fig. 2a). This behaviour is similar to that of other receptor-modulated adenylate cyclase systems¹⁷⁻¹⁹.

The following findings constitute additional evidence for the specificity and physiological relevance of the *in vitro* odorant response. (1) Odorants did not activate adenylate cyclase in membranes from unrelated tissues, for example, frog brain (Fig. 2b) or frog liver (not shown). (2) Although the odorants, either in mixture or individually applied, enhanced adenylate cyclase activity of olfactory cilia to various degrees, compounds known to be stimulatory in other membranes are ineffective (Fig. 1c). (3) The maximal *in vitro* odorant effect occurred at concentrations of organic compounds 10–100 times lower than those required for nonspecific modulation of adenylate cyclase via changes in membrane fluidity²⁸. (4) The odorant concentrations necessary to stimulate adenylate cyclase *in vitro* (a cell-popula-



tion response) correlated well with those known to give measurable *in vivo* summated electrophysiological recordings. Such recordings are typically carried out at 10^{-2} – 10^0 vapour saturation²⁵⁻²⁷ for odorous compounds respectively having vapour pressures of 5–0.05 mm Hg (at 25°C)^{25-27,29}. With molar water/air partition coefficients being in the range 1–1,000 (ref. 30), the physiological aqueous odorant concentrations would be 3×10^{-6} – 3×10^{-3} M, in good agreement with our experiments. (5) The low value (<0.5) of Hill slope for the *in vitro* odorant response (Fig. 2a) agrees well with the slopes of electrophysiological and psychophysical dose-response curves (see ref. 27). As similar *in vitro* values were also obtained for a single odorant (amyl acetate, not shown), the low Hill slope probably arises from receptor heterogeneity^{9,11,13,14}, which would also explain why saturation is not observed within the concentration range examined. Our finding that diverse ligands stimulate ciliary adenylate cyclase is also consistent with such receptor heterogeneity.

Inherent problems in the application of classical receptor binding techniques to olfactory research^{12,13} include the lack of defined pharmacological profiles and specific antagonists for individual receptor types, and the absence of 'negative controls' (odorant homologues that do not cause activation). These problems may at least partially be related to receptor heterogeneity, and could be alleviated by the future development of olfactory neuronal cell lines possessing defined odorant specificity¹⁴. Measurements of odorant-stimulated adenylate cyclase could help in the study of such cells.

The dependence of olfactory adenylate cyclase activity on guanine nucleotides suggests that a signal-transducing G-protein

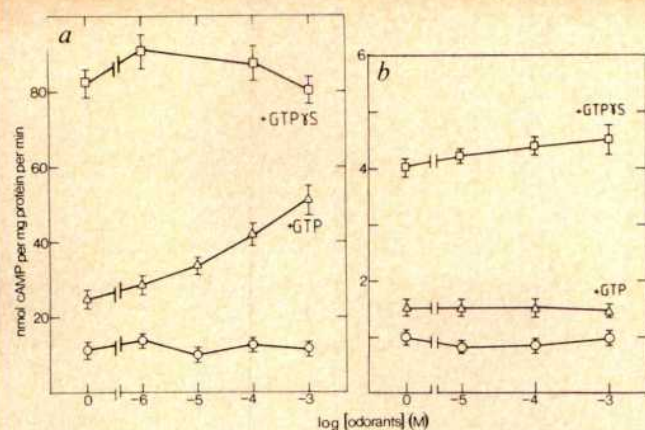


Fig. 2 Effect of odorants at increasing concentrations on adenylate cyclase activity in frog olfactory cilia (a) and frog brain membranes (b). Zero on abscissa means no odorants. GTP concentration is 10 μ M in both, GTP γ S concentration is 10 μ M in brain membranes and 1 nM in olfactory cilia. The lower curve in a and b is with no guanine nucleotides. Hill coefficients for the odorant response were calculated as the average slope of a graph of $\log Y/(1-Y)$ against $\log$ odorant concentration, where Y is the fraction of maximal stimulation attained. Maximal activation values from 55 to 80 nmol cyclic AMP per mg protein per min were used, to give a range of Hill coefficients of 0.35–0.49.

is involved, similar to the hormonal G-proteins^{17–19} and to visual transducin^{15,16}. To identify this protein, olfactory cilia were subjected to ³²P-ADP-ribosylation catalysed by bacterial toxins (Fig. 3). The cilia were found to contain a cholera toxin labelled polypeptide of relative molecular mass (M_r) 42,000 (42K) and a pertussis toxin labelled polypeptide of 40K. The first polypeptide has properties similar to those of the hormonal stimulatory G-protein (G_s), whereas the second is more like the inhibitory G-protein (G_i) or transducin^{15–19,31,32}. The polypeptide similar to G_s was found to be highly enriched in the cilia compared with membranes from deciliated epithelium, while the G_i -like protein is not (Fig. 3). This, together with the fact that odorants and GTP γ S increase rather than decrease ciliary adenylate cyclase activity, suggest that the G_s -like protein is the specific neuronal membrane component responsible for receptor-cyclase coupling. The possible slight difference in M_r between the ciliary and liver G_s (Fig. 3), and the observation (Fig. 2a) that olfactory adenylate cyclase can be fully activated by GTP γ S at much lower concentrations (1 nM) than those necessary for its hormonal counterpart (1–10 μ M, ref. 32), may imply that the olfactory G-protein is a distinct G_s variant.

Additional evidence for the involvement of a G-protein in olfactory reception could be obtained from studies of the interaction between the ligand-activated receptor and the ADP-ribosylation site on the G-protein, as shown for visual and some hormone receptors^{33,34}. Our preliminary evidence (U.P. and D.L., unpublished) suggests that the toxin-catalysed modification of the ciliary G-protein synergizes with odorants in the activation of adenylate cyclase. More research is needed to resolve this issue.

The present study provides a direct identification of molecular components involved in olfactory reception. The enzyme adenylate cyclase is shown to be present in high amounts in the olfactory neuronal dendrites, and its activity is specifically enhanced by functional ligands in a cell-free system. These results, together with the previously reported *in vivo* modulation of olfactory responses by cyclic AMP analogues and phosphodiesterase inhibitors^{21,22}, provide all the criteria²⁴ necessary for establishing the role of cyclic AMP as a second messenger in olfactory reception. Measurements of odorant-stimulated adenylate cyclase in olfactory cilia thus provide the first *in vitro* assay for olfactory reactivity, and could complement electrophysiological recordings in the study of odorant recognition.

Our results suggest that the putative olfactory receptor molecules^{6–14} activate a cyclic nucleotide modulating enzyme

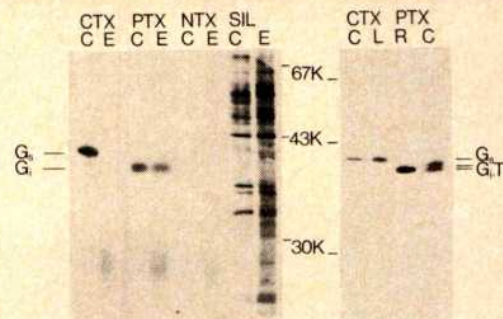


Fig. 3 SDS polyacrylamide gel electrophoresis autoradiography patterns of olfactory cilia (C), deciliated olfactory epithelial membranes (E), liver membranes (L) and retinal rod outer segments (R), all from frog, after ³²P-ADP-ribosylation in the absence (NTX) or presence of *Vibrio cholerae* toxin (CTX) or islet activating protein, *Bordetella pertussis* toxin (PTX) (both from List Biological Laboratories, Campbell, California). Silver staining patterns (SIL) are also shown, to indicate the relative amount of material used for labelling. ADP-ribosylation was carried out by a procedure similar to that of Schleifer *et al.*³⁹. Membranes (200 μ g ml⁻¹) were reacted at 37 °C in 20 mM Tris-HCl (pH 7.6) in the presence of 30 mM thymidine, 1 mM ATP, 0.1 mM GTP, 5 mM MgCl₂, 1 mM EDTA, 1 mM dithiothreitol (DTT), 5 μ M ³²P-NAD⁺ (NEN, 10–20 Ci mmol⁻¹), 3 mM phosphoenolpyruvate, 5 U ml⁻¹ pyruvate kinase, 0.05% Triton X-100. The reaction was stopped after 1 h by adding electrophoresis sample buffer and boiling. The toxins (10 μ g ml⁻¹) were activated by preincubation with 20 mM DTT at 37 °C for 15 min. M_r standards ($\times 10^{-3}$) are marked; G_s , G_i , the positions of the heavy (α) chain of the stimulatory and inhibitory G-proteins; T, heavy (α) chain of visual transducin. The left part of the figure is a more heavily labelled autoradiogram showing that, for comparable amounts of silver-stained protein, the cilia contain much more cholera toxin substrate than do epithelial membranes, but both have similar amounts of pertussis toxin substrate. No labelling is seen in either preparation when toxins are absent. The right part of the figure is lightly loaded and exposed, and is intended to provide a more accurate definition of the labelled polypeptides. Olfactory cilia have a single cholera toxin polypeptide substrate, which seems to co-migrate with a similarly labelled polypeptide in frog liver. Both migrate at about 0.85 of the distance between the carbonic anhydrase (M_r 31K) and ovalbumin (43K) standards, similar to the major cholera toxin substrate found in most other tissues and species^{18,40}. This polypeptide is named 42 K or 45K (we use the former) depending on the relative molecular mass assignment for the ovalbumin standard (43K or 45K–46K)⁴⁰. The olfactory preparations do not contain a 52K cholera toxin substrate, which is seen in some tissues and species and migrates more slowly than the ovalbumin standard⁴⁰. A weak band appearing below the 42K species in the leftmost lane may be a minor proteolytic cleavage product, and is seen occasionally in overloaded electrophoretograms. The pertussis toxin substrate of olfactory cilia is seen to be a doublet of polypeptide bands. The heavier component migrates between visual transducin and the G_i α -chains, and is putatively identified as the 40–41K polypeptide commonly ascribed to G_i α -chain^{18,40}. The lighter component almost co-migrates with transducin (39K). Multiple forms of pertussis toxin labelled polypeptides have been reported recently in nervous tissue^{41,42}.

via a GTP-binding signal-transducing protein. Olfaction thus appears to bear a striking molecular similarity to hormone, neurotransmitter and visual reception^{15–19}. This similarity can be used to clarify some aspects of olfactory function such as the role of transducing enzyme cascades in chemosensory signal amplification (see ref. 15). In parallel, olfaction may be viewed as a model for other molecular recognition processes. The fact that olfactory cilia preparations contain practically pure dendritic membranes derived from a single neuronal class, and are highly enriched in transducing components, make them suitable for studying general aspects of signal transfer in neuronal membranes.

The resemblance between olfaction and other transmembrane signalling mechanisms probably also applies to the receptor

molecules themselves. Thus, olfactory receptors may be integral membrane glycoproteins¹⁴ that interact with G-protein(s), as are the β -adrenergic receptor³⁵ or rhodopsin¹⁶. Such clues should be useful in devising new strategies for olfactory receptor studies. Accordingly, we are now examining the effect of lectins and monoclonal antibodies against defined ciliary surface glycoproteins^{11,36} on ciliary adenylate cyclase activity, and studying the direct association of glycoprotein receptor candidates with olfactory G-proteins.

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Vaccination with purified microgamete antigens prevents transmission of rodent malaria

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Malaria vaccination with preparations of microgametes has been shown to inhibit transmission of *Plasmodium* spp. to the mosquito vectors of avian^{1,2}, rodent³ and simian^{4,5} parasites. This transmission-blocking immunity results from the induction of microgamete-agglutinating antibodies in the vaccinated host which, when ingested in a mosquito blood meal, react with exflagellating microgametes in the midgut to prevent fertilization and oocyst development. Here we have passively transferred the immunity with gamete-specific monoclonal antibodies raised against the rodent malaria parasite *Plasmodium yoelii nigeriensis*⁶, and an IgG2a isotype monoclonal antibody from a hybridoma cell line, PYG-1, has been used to identify the target antigens on the gametes and to affinity-purify sufficient quantities of specific gamete antigen to facilitate vaccination studies. This transmission-blocking monoclonal antibody immunoprecipitated microgamete antigens of apparent relative molecular mass (M_r) 67K (67,000), 59K, 57K, 42K and 35K. Immunization of mice with these proteins before infection and mosquito feeding led to a 85-99.7% reduction in transmission to the mosquito vector; vaccination via intravenous or intramuscular routes was equally effective and did not require an adjuvant.

Gametocytes were purified from blood infected with *P. yoelii nigeriensis* by centrifugation through a discontinuous Percoll gradient⁷, washed and stimulated to undergo gametogenesis¹. After exflagellation, the soluble male gamete antigens were applied to an affinity column of transmission-blocking monoclonal antibody PYG.1 coupled to a Sepharose 4B matrix, washed through and the bound protein was eluted (see Fig. 1 legend).

Table 1 Vaccination with purified microgamete antigens prevents transmission of malaria

Group	Dose (μ g)	Route of administration	Mean no. oocysts per mosquito (range)	No. mosquitoes infected/total
1	40	i.v.	13 (0-60)	37/50
	40	i.m.	33 (0-120)	46/50
2	40, 30	i.v.	1 (0-7)	15/50
	40, 30	i.m.	2 (0-14)	18/50
Controls	—	—	350	60/60

Two groups of female BALB/c mice were injected i.v. or i.m. with 40 μ g of affinity-purified gamete antigen (see Fig. 1 legend). Two more groups of mice were immunized i.v. or i.m. with 40 μ g of soluble gamete antigen, followed 2 weeks later by a second dose of 30 μ g administered via the same route as the priming dose. All mice were infected i.v. with 10⁶ *P. y. nigeriensis*-infected erythrocytes 14 days after the final immunization. Three days after infection, batches of mosquitoes were fed for 30 min on each group of mice. Mosquitoes were maintained at a temperature of 24-27 °C for 1 week, before dissection and microscopic examination of their midguts for oocysts. All mosquitoes fed on control mice were heavily infected with oocysts; a random sample of 5 mosquitoes revealed a mean of 350 oocysts per mosquito.

SDS-polyacrylamide gel electrophoresis (PAGE) (Fig. 1) revealed that the monoclonal antibody immunopurified one major antigen of apparent M_r 42K and a series of minor proteins of M_r 67K, 50K, 57K and 35K. These proteins are specific to the sexual stage of the parasite; no bands were eluted from a PYG.1 column after application of solubilized asexual-stage parasites.

Female BALB/c mice were immunized as follows. Two groups of mice were each injected with a single 40- μ g dose of purified gamete antigen either intravenously (i.v.) or intramuscularly (i.m.), while two other groups of mice were immunized with 40 μ g of gamete antigen by the i.v. or i.m. route, followed 2 weeks later by a second dose of 30 μ g injected as per the priming dose.

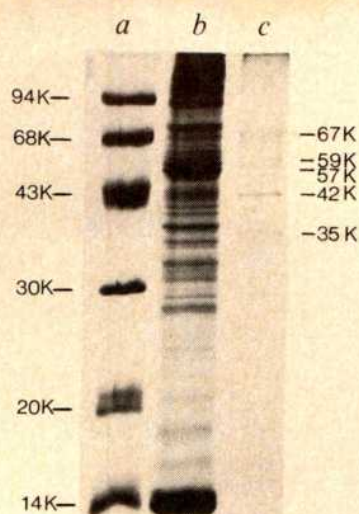


Fig. 1 SDS-PAGE analysis of *P. y. nigeriensis* gamete antigens. Lane a, M_r markers; lane b, total gamete antigen profile; lane c, PYG.1 affinity-purified gamete antigens.

Methods. Mouse blood with a gametocytaemia of 0.5–1.5% (parasitaemia 40–60%) was washed in suspended animation (SA) medium (0.21% Tris, 0.96% NaCl, 0.2% glucose, pH 7.4), in which gametogenesis is reversibly suppressed, and the gametocytes were purified by centrifugation through a discontinuous Percoll (Sigma) gradient as described in ref. 7. The gametocyte-enriched fraction was washed in SA medium and resuspended in gamete-releasing medium (2.5 vol. 5% NaCl, 2.5 vol 10% glucose, 20 vol 1.46% NaHCO_3 and 100 vol. heat-inactivated fetal calf serum adjusted to pH 8.0; ref. 1). After exflagellation, the male microgametes were separated by differential centrifugation and pelleted at 13,000g. Microgamete antigens were extracted on ice in 10 vol. of solubilizing medium (50 mM Tris pH 8.2 containing 1% Nonidet P-40, 5 mM EDTA, 5 mM EGTA, 5 mM iodoacetamide, 1 mM phenylmethylsulphonyl fluoride and 0.1 mM TLCK). Insoluble material was removed by centrifugation at 100,000g for 30 min at 4°C. The soluble gamete antigens were applied to an affinity column of transmission-blocking monoclonal antibody PYG.1 coupled to a Sepharose 4B matrix equilibrated with 10 mM Tris pH 8.0 containing 0.5 M NaCl and 5% (v/v) solubilizing medium. The column was washed with equilibrating buffer and the bound protein eluted with 50 mM diethylamine (pH 11.5) into tubes containing 1 M Tris pH 8.0. After neutralization, dialysis and concentration, the affinity-purified antigen was reappplied to the column and the affinity-purification procedure repeated. After further dialysis and concentration, the affinity-purified antigens were analysed by SDS-PAGE on 10% gels. Storage before use was at -30°C .

Sera taken from mice 10 days after a second i.v. or i.m. vaccination produced immunofluorescent staining patterns, using formalin-fixed whole gametes, which were indistinguishable from those produced by the monoclonal antibody PYG.1 (data not shown). Furthermore, using a combination of Western blot analysis and enzyme-linked immunosorbent assay techniques, these sera were found to react with a single gamete polypeptide of apparent M_r 42K, similar to the major protein in the affinity-purified gamete antigen preparation (Fig. 2), and thus confirming the immunogenicity of the affinity-purified antigen.

To determine whether immunization induced effective transmission-blocking immunity, all mice were injected i.v. with 10^6 *P. y. nigeriensis*-infected erythrocytes 14 days after the final immunization, and mosquitoes were fed on them 3 days later. The mosquitoes were maintained at 24–27°C for a further 7 days before dissection and enumeration of oocysts in the midguts. The results (Table 1) show that immunization with a single dose of antigen led, respectively, to a 95% (i.v. route) and 85% (i.m.) reduction in oocyst numbers relative to mosquitoes fed on unvaccinated animals. Animals given two doses

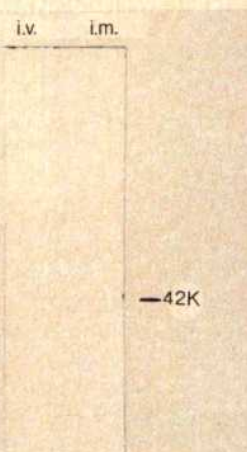


Fig. 2 Western blot analysis of *P. y. nigeriensis* gamete antigens. Solubilized unfractionated whole gamete proteins were separated by SDS-PAGE on a 10% gel and transferred electrophoretically to a nitrocellulose membrane. After blocking with 5% (w/v) bovine serum albumin in phosphate-buffered saline, the nitrocellulose membranes were incubated for 45 min with sera (diluted 1:100 in phosphate-buffered saline) taken from mice immunized i.v. or i.m. with affinity-purified gamete antigen. The membranes were washed and incubated with peroxidase-conjugated rabbit anti-mouse immunoglobulin for 45 min. The strips were then washed and stained with the substrate diaminobenzidine. The estimated M_r of the polypeptide is indicated.

of vaccine via the i.v. or i.m. routes were found to be almost totally uninfected to feeding mosquitoes, with oocyst numbers reduced by 99.7% and 99.3%, respectively. Indeed, more than two-thirds of mosquitoes in the latter groups were found to be uninfected. The transmission-blocking immunity was stage-specific in that no significant protection against the asexual erythrocytic forms of the disease was noted in any of the vaccinated animals (data not shown).

Although it is well documented that specific monoclonal antibodies directed against the sexual stages of *Plasmodium* will effectively block transmission of the parasite to its mosquito vector^{6,8–10}, this is the first report of purified soluble gamete antigens inducing an effective transmission-blocking immunity. The immunity is potent and does not require the co-administration of an adjuvant.

The mechanisms by which the host immune system mediates suppression of transmission appears to be serum-mediated (Fig. 2), as suggested by others^{2,6,10}. Microgametes are only susceptible to the action of the immune system immediately after exflagellation, and it is likely that the antibodies raised against the soluble antigen act to agglutinate gametes in the mosquito gut, preventing fertilization and consequently the transmission of infection.

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A uniform deleting element mediates the loss of κ genes in human B cells

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Human immunoglobulin light-chain genes become rearranged in an ordered fashion during pre-B-cell development such that rearrangement generally occurs in κ genes before λ genes (refs 1, 2). This ordered process includes an unanticipated deletion of the constant κ (C_κ) gene and κ enhancer sequence which precedes λ rearrangement¹⁻⁴, and the site of this deletional recombination was located 3' to the joining (J_κ) segments in 75% of cases studied. We have now characterized the recombinational element responsible for this event on three separate alleles and found them to be identical. This κ -deleting element recombined site-specifically with a palindromic signal (CACAGTG) located in the J_κ - C_κ intron. All losses of C_κ genes in other human B cells were mediated by this determinant, including the 25% of instances when this element recombined with sequences 5' to J_κ . In contrast, the κ -deleting element remained in its germline form on all successful κ -producing alleles. Moreover, κ loss is an evolutionarily conserved event, as the κ -deleting element appears to be the human homologue of the murine RS sequence⁵. Our results suggest that this element may help ensure isotypic and allelic exclusion of light chains and may be involved in the ordered use of human light-chain genes.

The two available light-chain classes in humans are used almost equally (κ , 60%; λ , 40%), yet the gene configuration of the excluded light-chain class in κ - and λ -producing B cells shows a marked dichotomy. Clonal cell lines or leukaemias of κ -producing B cells usually retain germline λ genes. In contrast, λ -producing B-cell lines have no remaining germline κ genes, at least the C_κ portion having usually been deleted³. Deletion of the κ genes is a normal developmental event unrelated to transformation⁶, and our examination of B-cell precursors indicated that this deletional event can occur before λ gene rearrangement^{1,2,4}. We further investigated the extent of the κ deletion and found that the C_κ region and the κ enhancer element

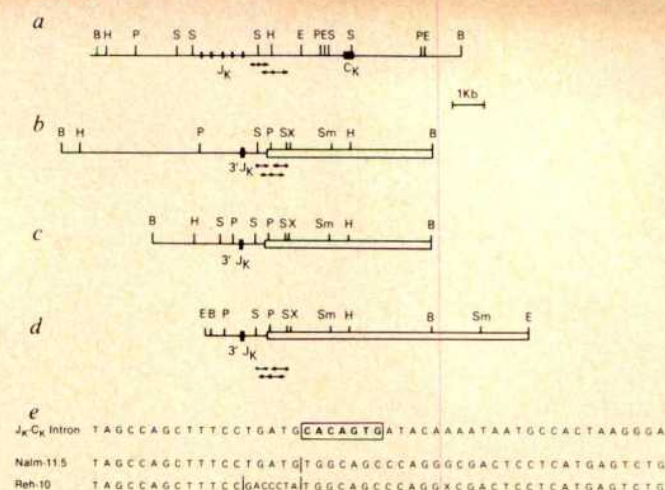


Fig. 2 Restriction map analysis, sequencing strategy and nucleotide sequence of the germline J_κ - C_κ region and three rearranged alleles bearing the 3' rearrangement. **a**, The germline J_κ - C_κ region restriction map in which arrows indicate sequencing strategy for M13 subclones sequenced by the Sanger dideoxy termination method³⁰. Three rearranged alleles (**b**, **c**, **d**) were cloned from *Bam*HI-digested (**b**, **c**) or *Eco*RI-digested (**d**) genomic DNA that was size-selected by preparative gel electrophoresis, ligated into the phage vectors Charon 28 or Charon 4A, and successfully screened with a J_κ segment probe^{25-27,31}. **b** is an 11.5-kb *Bam*HI J_κ -containing allele, **c** is an 8.8-kb *Bam*HI J_κ -containing allele from Nalm-6, while **d** is a 10-kb *Eco*RI J_κ -containing allele from Reh. In all three alleles a uniform κ -deleting element (κ de) has been introduced at their 3' end (open box), and all have undergone a separate rearrangement on the 5' side of J_κ . Nucleotide sequence analysis revealed the palindromic heptamer (CACAGTG) at 917 base pairs 3' to J_κ 5 in the germline J_κ - C_κ intron (**e**). The nucleotide sequence of the introduced κ de is identical in the Nalm-11.5 and Reh-10 alleles. Their recombination breakpoints are focused around the palindromic sequence. H, *Hind*III; B, *Bam*HI; P, *Pst*I; S, *Sac*I; E, *Eco*RI; X, *Xho*; Sm, *Sma*I.

were uniformly lost from the genome in all 20 instances examined (Fig. 1, Table 1). This was true for all deletions of κ genes in λ -bearing B cells, pre-B cells and those instances in which the excluded κ allele in κ -producing B cells was lost (Fig. 1, Table 1). All J_κ segments were also deleted in 5 of 20 instances, but were retained in a rearranged state in 15. Previous

Fig. 1 Deletions of J_κ and C_κ regions in human B cells. Representative genomic blots are presented of *Bam*HI-digested DNA which was electrophoresed in parallel on the same agarose gel, transferred to nitrocellulose paper, then hybridized with the indicated probes²⁵⁻²⁷. A human placenta served as a germline control, LY67 is a μ , λ -producing Burkitt lymphoma cell line²⁸, Nalm-6 and Reh are B-cell precursor acute lymphoblastic leukaemia cell lines² and SU-DHL-6 is a μ , κ -producing diffuse histiocytic lymphoma cell line²³. A 2.5-kb *Eco*RI genomic fragment was used as a C_κ probe and a 1.8-kb *Sac*I germline genomic fragment was used as a J_κ probe²⁹. Dash marks indicate the germline position and arrows denote rearrangements. A 0.7-kb *Eco*RI fragment containing the enhancer sequence revealed that the κ enhancer region was also deleted each time C_κ was lost (data not shown). Cytogenetic analysis of these cell lines revealed no translocations or obvious deletions of the short arm of chromosome 2. Asterisks indicate probes used in hybridization.

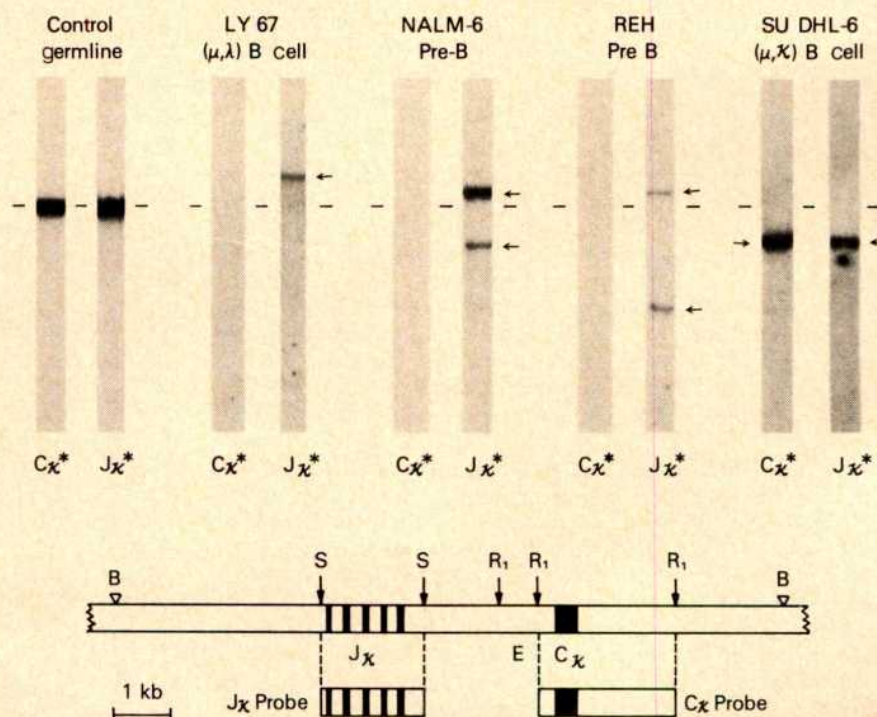


Table 1 The configuration of both κ gene alleles in 12 additional cell lines and leukaemias at different stages of B-cell development

Cell identification	Configuration of the κ alleles in		
	<i>Bam</i> HI genomic digests with probes of:		
Pre-B cells	$C_{\kappa}^*E_{\kappa}^*$	J_{κ}^*	κde^*
Nall-1	Deleted ($E_{\kappa}-C_{\kappa}$) Deleted ($E_{\kappa}-C_{\kappa}$)		Rearranged ($J_{\kappa}/\kappa de$) Rearranged ($J_{\kappa}/\kappa de$)
ALL-RT-5		Rearranged ($V/J_{\kappa}-C_{\kappa}$) Deleted ($J_{\kappa}-C_{\kappa}$)	Germline κde Rearranged (5' sequence/ κde)
RS 4; 11		Rearranged ($V/J_{\kappa}-C_{\kappa}$) Deleted ($J_{\kappa}-C_{\kappa}$)	Germline κde Rearranged (5' sequence/ κde)
κ -Producing B cells			
CLL-RT		Rearranged ($V/J_{\kappa}-C_{\kappa}$) Rearranged ($V/J_{\kappa}-C_{\kappa}$)	Germline κde Germline κde
SU-DHL-4		Rearranged ($V/J_{\kappa}-C_{\kappa}$) Germline $J_{\kappa}-C_{\kappa}$	Germline κde Germline κde
BHM23		Rearranged ($V/J_{\kappa}-C_{\kappa}$)	Germline κde
SU-DHL-7	Deleted ($E_{\kappa}-C_{\kappa}$)	Rearranged ($J_{\kappa}/\kappa de$)	Germline κde Germline κde
λ -Producing B cells			
SU-DHL-5	Deleted ($E_{\kappa}-C_{\kappa}$)	Rearranged ($J_{\kappa}/\kappa de$) Deleted ($J_{\kappa}-C_{\kappa}$)	Rearranged (5' sequence/ κde)
RPMI 8392	Deleted ($E_{\kappa}-C_{\kappa}$) Deleted ($E_{\kappa}-C_{\kappa}$)	Rearranged ($J_{\kappa}/\kappa de$) Rearranged ($J_{\kappa}/\kappa de$)	Rearranged ($J_{\kappa}/\kappa de$) Rearranged ($J_{\kappa}/\kappa de$)
CLL-DG	Deleted ($E_{\kappa}-C_{\kappa}$) Deleted ($E_{\kappa}-C_{\kappa}$)	Rearranged ($J_{\kappa}/\kappa de$) Rearranged ($J_{\kappa}/\kappa de$)	Rearranged ($J_{\kappa}/\kappa de$) Rearranged ($J_{\kappa}/\kappa de$)
Non-producing B cells			
SU-DHL-2		Rearranged ($V/J_{\kappa}-C_{\kappa}$) Rearranged ($V/J_{\kappa}-C_{\kappa}$)	Germline κde Germline κde
VDS		Rearranged ($V/J_{\kappa}-C_{\kappa}$) Deleted ($E_{\kappa}-C_{\kappa}$)	Germline κde Rearranged ($J_{\kappa}/\kappa de$)

We examined three additional pre-B cells, Nall-1, ALL-RT-5 (ref. 2) and RS 4; 11 (ref. 22); four κ -producing B cells, CLL-RT, SU-DHL-4 (ref. 23), BHM23, SU-DHL-7 (ref. 23); three λ -producing B cells, SU-DHL-5 (ref. 23), RPMI 8392 (ref. 24) and CLL-DG; and two non-producing B-cell lines, SU-DHL-2 (ref. 23) and VDS. *Bam*HI-digested genomic DNA from individual cells was electrophoresed in parallel on the same gel and then hybridized to the previously described C_{κ}^* and J_{κ}^* probes, to a 0.7-kb *Eco*RI fragment containing the enhancer sequence (E_{κ}^*)¹⁷, and to the 2.5-kb *Hind*III/*Bam*HI κ -deleting element (κde^*) probe. Whenever the enhancer to C_{κ} ($E_{\kappa}-C_{\kappa}$) regions were deleted, the J_{κ} segments were found to be rearranged onto the same *Bam*HI fragment as the κde ($J_{\kappa}/\kappa de$). When the J_{κ} , E_{κ} and C_{κ} regions ($J_{\kappa}-C_{\kappa}$) were deleted, the κde was always rearranged to sequences apparently 5' to J_{κ} (5' sequence/ κde). In contrast all alleles with rearrangements limited to the 5' side of J_{κ} retained the E_{κ} and C_{κ} regions and were presumably attempts at V/J joining ($V/J_{\kappa}-C_{\kappa}$). These rearrangements always retained a germline κde .

studies indicated that one variable-region (V_{κ}) gene family was retained in its germline state³. We reasoned that cells in which the J_{κ} region was rearranged while the enhancer and C_{κ} region were deleted represented an opportunity to identify a 3' element that was being introduced by this deletion recombination. Therefore, we cloned the rearranged J_{κ} alleles from the B-cell precursor lines Nalm-6 and Reh.

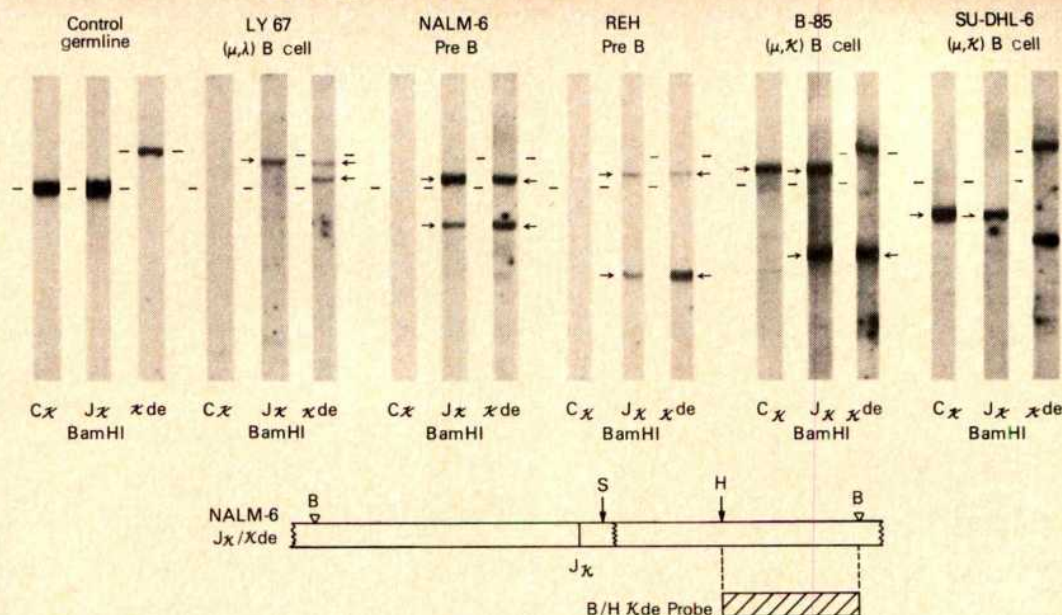
Both rearranged J_{κ} alleles from Nalm-6 and one rearranged J_{κ} allele from Reh were isolated from these pre-B-cell lines and compared with the germline $J_{\kappa}-C_{\kappa}$ region (Fig. 2). Restriction map analysis indicated that all three J_{κ} alleles had undergone a 5' rearrangement, presumably from attempts at V/J joining^{7,8}. Furthermore, all three J_{κ} alleles had undergone a 3' rearrangement which introduced a uniform element into the $J_{\kappa}-C_{\kappa}$ intron. DNA sequence analysis of the new 3' element from both cell lines confirmed that they were identical (Fig. 2). We term this introduced sequence the κ -deleting element (κde). As these three examples of a rearranged κde seemed to be recombining within a limited region of the $J_{\kappa}-C_{\kappa}$ intron (Fig. 2), we sequenced this previously uncharacterized portion of the germline $J_{\kappa}-C_{\kappa}$ intron, searching for a site-specific target for recombination. At 917 base pairs 3' to the last J_{κ} segment ($J_{\kappa}5$), we noted the palindromic heptanucleotide (CACAGTG). Interestingly, this is identical to the heptamer flanking germline V and J regions which is presumably involved in V/J joining⁹⁻¹¹. The Nalm-11.5 allele rearranged precisely at this palindrome and the Reh-10 allele rearranged at or near it (Fig. 2). This suggests that the introduction of the κde into the $J_{\kappa}-C_{\kappa}$ intron uses a site-specific recombination mechanism.

We found that this uniform κ -deleting element mediated the

loss of C_{κ} regions in all other instances that we examined. A 2.5-kilobase (kb) *Hind*III/*Bam*HI fragment prepared from the κde of Nalm-11.5 recognized a unique 14-kb *Bam*HI genomic fragment in its germline form (Fig. 3). The germline position of the deletion element is thus clearly separate from the germline $J_{\kappa}-C_{\kappa}$ *Bam*HI fragment. Figure 3 and Table 1 indicate that there is a rearrangement of this κde on every allele in which C_{κ} is deleted. In all instances in which C_{κ} is deleted but the J_{κ} segment is retained, the κde can be shown to co-occupy the same *Bam*HI restriction fragment with the J_{κ} segment. This is true not only in Nalm-6 and Reh, from which the κde was cloned, but also for 12 other alleles (Fig. 3, Table 1). Furthermore, one allele of the κde is always rearranged in those instances in which the J_{κ} as well as the enhancer and C_{κ} segment are deleted. One allele in LY67 and SU-DHL-6 demonstrate this in Fig. 3, and further examples are noted in Table 1. This indicates that the κde has a target site(s) 5' to J_{κ} as well as within the $J_{\kappa}-C_{\kappa}$ intron. In contrast, κ -producing cell lines always retain a germline configuration of the κde on the expressed allele, as illustrated by B-85, SU-DHL-6, CLL-RG, SU-DHL-4, BHM23 and SU-DHL-7 (Fig. 3, Table 1). In approximately 25% of cases, even κ -producing B cells display a deletion of the phenotypically excluded κ allele. In these cells, as shown by B-85, SU-DHL-6 and BHM23, the κde is also responsible for the elimination of κ genes. Thus, this same κde accounts for all losses of C_{κ} genes observed in pre-B cells, λ -producing B cells and κ -producing B cells.

Rearrangement and elimination of κ genes in λ -producing B cells is an evolutionarily conserved event^{5,12-14}. Even though the mouse expresses λ on only 5% of its B cells, the C_{κ} regions of

Fig. 3 Rearrangement of the κ -deleting element (κ de) in human B cells. Representative genomic blots of co-migrated *Bam*HI-digested DNA that was hybridized with the C_{κ} , J_{κ} and enhancer (E_{κ}) probes as well as a 2.5-kb *Hind*III/*Bam*HI fragment of the κ de (cross-hatched bars) from the 11.5-kb allele of Nalm-6. Dash marks indicate the germline position and arrows denote rearrangements. On alleles in which only the C_{κ} and E_{κ} regions have been deleted, the J_{κ} region co-occupies a *Bam*HI fragment with the rearranged κ de (LY67, Nalm-6, Reh). On alleles in which the entire J_{κ} - C_{κ} region is deleted, the κ de is also rearranged (LY67, SU-DHL-6). On the productive κ -producing allele of B-85 and SU-DHL-6, the J_{κ} and C_{κ} segment co-occupy a rearranged *Bam*HI fragment, but the κ de remains germline.



these cells have frequently been deleted. Durdik *et al.* have identified an element that recombines with the identical conserved palindrome (CACAGTG) within the J_{κ} - C_{κ} intron of the mouse⁵. This is the same intron heptamer that underwent a 5' rearrangement with a V_{κ} region in MPC11, resulting in a truncated κ -chain product^{15,16}. However, the major role of this signal appears to be as an acceptor site for the κ de, resulting in the removal of the C_{κ} and enhancer region. This heptamer also flanks germline V and J regions, suggesting that the recombinatorial enzymes that help activate this gene (V/J joining) also mediate its destruction.

The use of a κ -deleting element in two species suggests strongly that it has a functional role. By removing the κ possibility, the κ de might ensure λ gene utilization. Moreover, all κ de rearrangements result in the elimination of the κ enhancer element^{17,18}. Studies with a pSV2-CAT construct have suggested that only a limited quantity of a *trans*-acting factor recognizing that enhancer exists in host cells¹⁹. Thus, the deletion of the κ enhancer may transfer the effect of an immunoglobulin enhancer-specific factor to a putative enhancer within the λ locus^{20,21}. Alternatively, either the germline or rearranged κ de may itself encode a *trans*-acting factor that affects λ gene rearrangement or transcription. The κ -deleting element may therefore mediate the ordered use of light-chain genes and help ensure that B cells make only one light chain of a selected light-chain class.

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A molecular clone of HTLV-III with biological activity

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Acquired immune deficiency syndrome (AIDS) is an epidemic immunosuppressive disease characteristically associated with a depletion of T lymphocytes of the helper/inducer phenotype¹. Numerous converging lines of research have implicated a human T-cell lymphotropic retrovirus, HTLV-III, in the pathogenesis of AIDS²⁻⁵. Recently, several distinct forms of the HTLV-III genome were molecularly cloned in phage and extensively characterized^{6,7}. In the present study, a clone containing full-length HTLV-III proviral DNA⁷ was inserted into a plasmid and used to transfect cord blood T cells from normal newborn humans. We demonstrate that this molecular clone is infectious *in vitro* and causes marked cytopathic effects on T-cell cultures. This is the first direct evidence that the HTLV-III genome, rather than a minor component of the virus complex, is cytopathic for T cells. Using this biologically competent clone and mutants derived from it, it should now be possible to localize the subgenomic regions that contribute to the biological effects of HTLV-III.

Studies of cell-mediated immunity in AIDS victims reveal a generalized impairment of T-lymphocyte function accompanied by a reduction in T lymphocytes bearing the OKT4 antigen¹. The knowledge that a retrovirus (feline leukaemia virus) is often associated with severe immunodeficiency in cats⁸, and that the known human retroviruses are T-cell tropic and can impair immune function (see ref. 9 for a review), prompted speculation that a T-cell lymphotropic retrovirus might be the causal agent of AIDS. Two groups of human T-cell lymphotropic viruses,

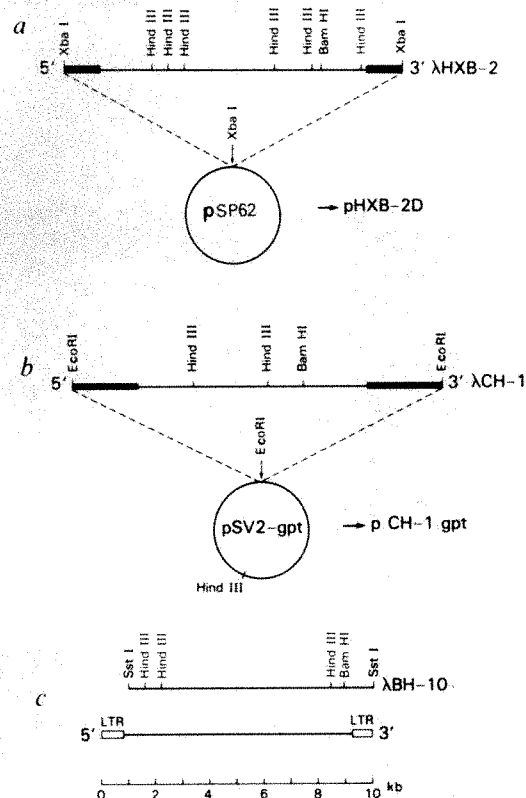


Fig. 1 Construction of plasmids containing HTLV-III or HTLV-I proviral sequences. A 12.7-kb Xba I fragment derived from λ HXB-2D, a molecular clone containing ~ 10 kb of HTLV-III proviral sequences, was inserted into the Xba I site in the polylinker of plasmid pSP62²⁸. Similarly, a 13.7-kb Eco RI fragment of λ CH-1 (a molecular clone containing ~ 9.0 kb of HTLV-I proviral sequences)²⁹ was inserted into the Eco RI site of pSV2gpt³⁰. These plasmid constructs were then transfected into DH-1 bacteria and used in protoplast fusion experiments. The thin horizontal line represents HTLV-III (a) or HTLV-I (b) sequences; the solid boxes represent flanking cellular sequences; and the open boxes in c represent the long terminal repeat (LTR) regions of the molecular clone λ BH-10. BH-10 is an incomplete viral clone of HTLV-III which lacks a 190-base pair segment in the 5'-LTR and leader sequence⁷. Insert from this clone was used to prepare the probe for hybridization analyses.

HTLV-I and HTLV-II, have been characterized and their association with T-cell malignancies reported^{10,11}. Recently, a third distinct group of retroviruses has been repeatedly isolated from patients with AIDS or from those at risk for the disease^{3,12,13}. These retroviruses, designated by different groups as human T-cell lymphotropic virus type III (HTLV-III)³, lymphadenopathy-associated virus (LAV)¹², and AIDS-related virus (ARV)¹³, are variants of the same virus as shown by DNA sequence analyses¹⁴. Our laboratory has shown that circulating antibody to HTLV-III can be detected in sera from over 90% of AIDS patients and from a high proportion of people in various high-risk groups, but not from sera of healthy people in non-risk groups⁵. These results, together with demonstrations that HTLV-III has a cytopathic effect on OKT4-positive (OKT4⁺) cells¹⁵, clearly implicate HTLV-III in the aetiology of AIDS.

HTLV-III has many properties in common with HTLV-I and HTLV-II, including a propensity to infect OKT4⁺ lymphocytes, a reverse transcriptase that prefers Mg^{2+} , a major core protein of relative molecular mass 23,000–25,000 and immunological relatedness to some of the major viral proteins⁴. In addition, these viruses have in common the property of *trans*-acting transcriptional activation, probably mediated by a unique viral gene (*lor*) product^{16,17}. However, HTLV-III differs strikingly from other HTLV members in at least two important aspects: (1) HTLV-III is highly replicative *in vivo* and shows considerable genomic diversity⁷, and (2) normal T cells infected with

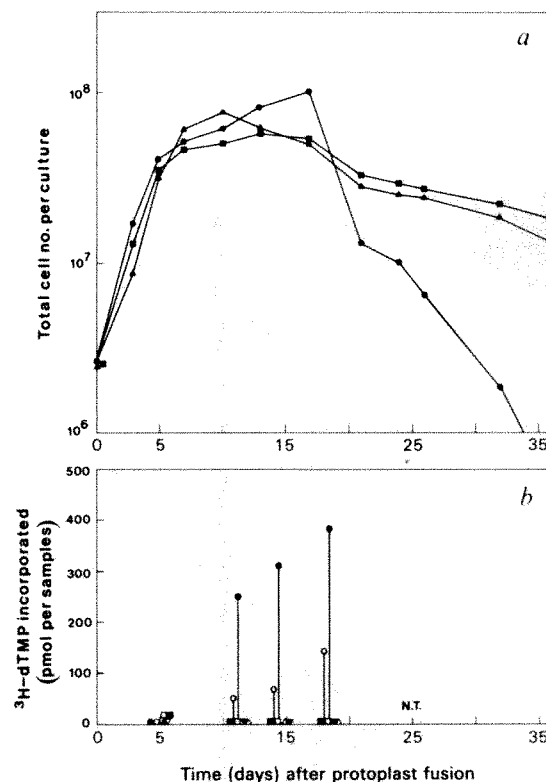


Fig. 2 Kinetics of cell growth and reverse transcriptase activity in cord blood mononuclear cell cultures following protoplast fusion. Mononuclear cells were prepared from cord blood samples using Ficoll Triosil and cultured for 5 days in media containing PHA. These cells were then fused with bacterial protoplasts carrying the plasmid pHXB-2D ($\circ/\bullet$), pSV2neo ($\Delta/\blacktriangle$) or pCH-1gpt ($\square/\blacksquare$) and maintained in culture at a density of 5×10^5 cells ml^{-1} by addition of RPMI-1640 medium containing 20% fetal calf serum, 10% T-cell growth factor (interleukin-2) and antibiotics. Three parallel fusions using cells from different individuals were established for each plasmid. In a, the growth of the transfected cells is expressed as the mean value for three fusion experiments. Spent medium removed from two cultures at 5, 11, 14 and 18 days after fusion was concentrated 10-fold and assayed for the presence of reverse transcriptase using standard techniques³¹. The activity detected in each of the culture supernatants is expressed (b) as the amount of ³H-labelled deoxyribonucleoside monophosphate (³H-dTMP) incorporated (in pmol per 0.3-ml sample) using dT₁₅·(rA)_n as the template primer.

Methods. Protoplast fusion: Single colonies of bacteria bearing the plasmid of interest were grown in L-broth containing ampicillin to a density of $2-4 \times 10^8$ ml^{-1} and chloramphenicol was added to a final concentration of 250 μg per ml of broth. After incubation at 37 °C for 2 h, gentamycin was added to 10 μg ml^{-1} and the cultures were re-incubated for 14–18 h to allow plasmid amplification. The bacteria were collected and resuspended in 2.5 ml of HEPES-buffered saline (HBS) pH 8.0, containing 20% sucrose. This suspension was placed on ice and at 5-min intervals, 0.8 ml of lysozyme solution (10 mg ml^{-1} in 0.25 M Tris-HCl pH 7.0), 1.0 ml of 0.25 M EDTA pH 8.0, and 1.0 ml of HBS were added sequentially. The suspension was incubated at 37 °C for 10–30 min until >90% of the bacteria had formed protoplasts, then held on ice and slowly diluted to 50 ml with RPMI-1640 medium. For each fusion, 10^{10} bacterial protoplasts were combined with 2.5×10^6 PHA-stimulated core blood cells, washed and the pellet resuspended in 0.1 ml of RPMI-1640 medium. To these cells, 0.8 ml of polyethylene glycol fusion reagent (PEG-FR) was added dropwise over 1 min, the cells were left to stand for a further minute and then gradually diluted with RPMI-1640. PEG-FR was prepared as a 48% solution in RPMI-1640 medium and was pretreated by passing it over a resin bed²². After fusion, the cells were washed and returned to culture.

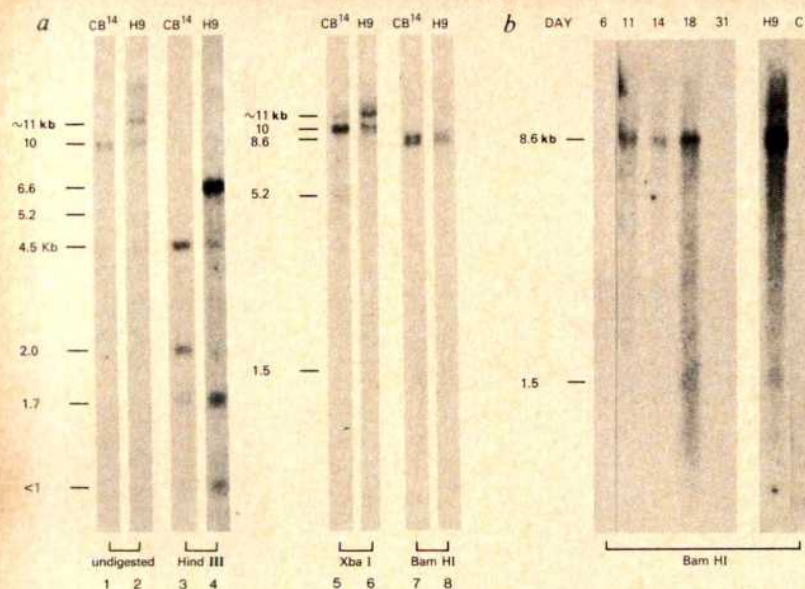


Fig. 3 Demonstration of the presence of HTLV-III sequences in cord blood cell cultures transfected with pHXB-2D. **a**, DNA of high relative molecular mass was isolated from H9/HTLV-III_B cells (H9) and from cord blood cell cultures 14 days after transfection with pHXB-2D (CB¹⁴) using standard protocols³². H9/HTLV-III_B is a permanent human T-cell line which continuously produces large quantities of HTLV-III. DNA (5 µg) was either left undigested (lanes 1 and 2) or digested separately with the enzymes *Hind*III (lanes 3, 4), *Xba*I (5, 6) or *Bam*HI (7, 8), electrophoresed in standard 0.8% agarose gels, blotted and hybridized to probe prepared from nick-translation of the BH-10 insert. The filters were washed in 0.5×SSC, 0.1% SDS at 65°C for 4–6 h before exposure. **b**, A time-course study in which DNA from cord blood cells transfected with pHXB-2D was analysed for HTLV-III sequences 6, 11, 14, 18 and 31 days after protoplast fusion. DNA (5 µg) was digested with *Bam*HI and hybridized to the BH-10 probe (under the conditions described for **a**). DNAs prepared from H9/HTLV-III (H9) and from PHA-stimulated cord blood cell cultures (C) were included as positive and negative controls, respectively.

HTLV-III degenerate rapidly in culture. In contrast, although HTLV-I and HTLV-II exert some cytopathic effects, they are able to efficiently immortalize some of the infected T cells in *in vitro* co-culture^{18–20} and in cell-free²¹ transmission studies. The mechanism by which HTLV abrogates normal T-cell function, leading to transformation (in the case of HTLV-I/ HTLV-II) or death (in the case of HTLV-III), is unknown.

In the present study, we used a transfection technique to investigate the biological properties of molecularly cloned HTLV-III DNA. The phage clone λHXB-2D, which contains full-length integrated provirus (~10 kilobases, kb) with cellular flanking sequences (12.7 kb total length), was recently isolated in our laboratory from an H9/HTLV-III genomic library constructed using *Xba*I⁷. We inserted the 12.7-kb *Xba*I fragment from this clone into plasmid pSP62 (Fig. 1) and introduced this construct into phytohaemagglutinin (PHA)-stimulated lymphocytes using the protoplast fusion technique²². Plasmids containing full-length HTLV-I (pCH-Igpt) or no HTLV sequences (pSV2neo) were used as controls (Fig. 1).

Figure 2a summarizes the growth kinetics of T-cell cultures transfected with pHXB-2D, pSV2neo or pCH-Igpt. The growth of all cultures was comparable for the first 14 days after protoplast fusion. By day 18, however, the number of viable cells in cultures transfected with pHXB-2D had fallen dramatically; there was a 10-fold and a 100-fold reduction between days 18 and 21 and 18 and 32, respectively. Cultures transfected with either pSV2neo or pCH-Igpt showed only a 4–5-fold reduction over the same time period. When supernatant from the cultures was assayed for the presence of reverse transcriptase, activity was detected exclusively in cultures transfected with pHXB-2D (Fig. 2b). These data suggest that replicating virus was present in cultures 11–18 days after fusion with pHXB-2D protoplasts. The HTLV-I clone (pCH-Igpt) was apparently biologically inactive.

The presence of HTLV-III sequences in the transfected cells was demonstrated by Southern blot analysis (Fig. 3). DNA isolated from H9/HTLV-III_B was used as a positive control (H9). A 10-kb band, corresponding to unintegrated linear virus, was detected in undigested DNA samples prepared 14 days after transfection (Fig. 3a, lane 1). Digestion with *Xba*I revealed three distinct bands at 11, 10 and 5.2 kb; these co-migrate with bands frequently seen in undigested DNA from HTLV-III-infected H9 cells and other HTLV-III-infected cell lines and fresh tissues⁷ (Fig. 3a, lane 2). As *Xba*I does not cut within the viral sequences of clone pHXB-2D, these bands probably represent the nicked circular, linear and closed circular forms of unintegrated HTLV-III, respectively. In the *Xba*I-digested DNA samples, there was an increase in intensity of the nicked circular form compared with that in the undigested DNA sample (Fig.

3a, compare lanes 5 and 1); this was probably a result of nonspecific cleavage during incubation with the enzyme. The failure to demonstrate a DNA fragment of 12.7 kb shows that there was little or no plasmid carrying HTLV-III sequences in these cultures.

Digestion with *Hind*III, an enzyme which cuts the HTLV-III genome of pHXB-2D six times, yielded bands at 4.5, 2.0 (a doublet), 1.7 and 0.6 kb (a doublet) (Fig. 3a, lane 3), consistent with the fragments expected to be generated by *Hind*III digestion of linear unintegrated virus. This restriction pattern is clearly different from that of H9/HTLV-III_B (Fig. 3a, lane 4). The H9/HTLV-III_B cell line was derived from the human T-cell line HT, following co-culture with T lymphocytes obtained from several AIDS patients³, and contains many different HTLV-III forms. Thus, the differences in restriction patterns are consistent with H9/HTLV-III_B containing, as a minor component, a provirus equivalent to that of pHXB-2D. The major component of H9/HTLV-III differs, however, from the HTLV-III genome of pHXB-2D by the absence of a *Hind*III site ~3.5 kb from the 3' end of the virus⁷. *Bam*HI digestion of the DNA from the transfected cultures gave two fragments (8.6 and 1.5 kb) which result from a single cut of the linearized genome (Fig. 3a, lane 7). High-relative molecular mass 'smears' were not observed when DNA was digested with *Bam*HI. Therefore, we have no direct evidence that transfected HTLV-III DNA is integrated in the host cell genome. HTLV-III may integrate randomly and at too low a frequency to be detected by Southern blotting. Alternatively, it is conceivable that HTLV-III, like spleen necrosis virus and visna virus (cytopathic avian and ovine retroviruses), may not need to be integrated for virus production^{23,24}.

Expression of the HTLV-III gag-related proteins p15 and p24 by transfected cells was demonstrated using specific monoclonal antibodies (Fig. 4a–e): maximum expression was observed 18 days after transfection, when 4–11% and 5–9% of cells were reactive with antibody to p15 and p24, respectively (data not shown). Virus particles were detected by electron microscopy in all cultures 14–18 days after transfection with pHXB-2D (Fig. 4f). The particles contained condensed, truncated cores, which are characteristic of HTLV-III particles²⁵.

In time-course experiments (Fig. 3b), DNA isolated from a single culture 6, 11, 14, 18 and 31 days after transfection with pHXB-2D, was digested with *Bam*HI and analysed for HTLV-III sequences. Six days after transfection, an 8.6-kb DNA fragment was detected as a faint band; 18 days after transfection it was possible to detect a 1.5-kb DNA fragment in addition to the 8.6-kb fragment (Fig. 3b). The total amount of unintegrated virus in the cultures appeared to increase, as suggested by the increase in intensity of these bands with time; we interpret this as evidence that cells originally transfected with pHXB-2D are

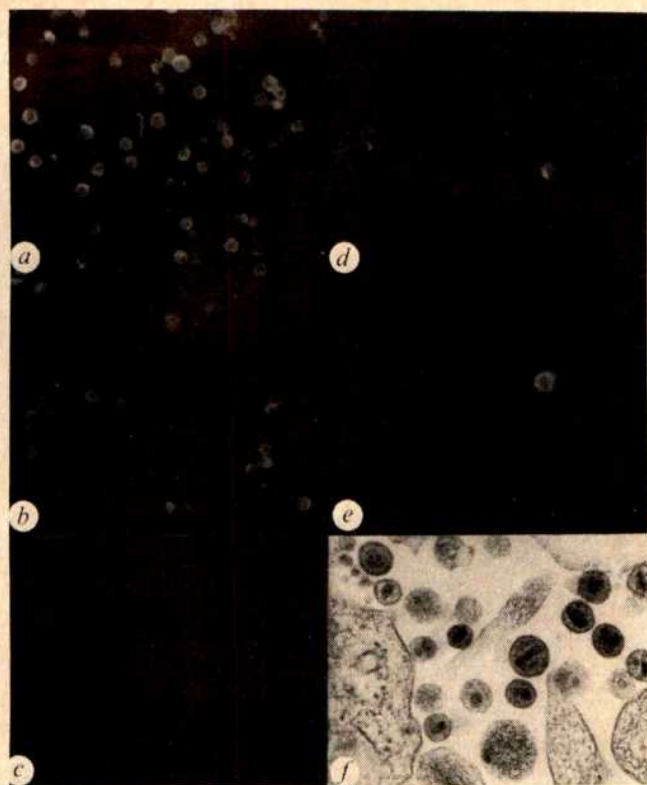


Fig. 4 Expression of HTLV-III p15 and p24 antigens and the production of virus particles by cord blood cell cultures transfected with pHXB-2D. Indirect immunofluorescence studies were performed using monoclonal antibodies against the HTLV-III gag-related proteins p24 (a, d) and p15 (b, e) (ref. 33 and A. Bodner, unpublished observations). Cells were fixed for 5 min using a 1:1 mixture of acetone/methanol and standard techniques. d, e, Cord blood cells removed from culture 18 days after transfection with pHXB-2D and stained to reveal the binding of these antibodies. A small percentage (4–11%) of the cells clearly express HTLV-III p24 and p15 antigens. In comparison, among H9/HTLV-III cultures (a, b), a much larger proportion of cells (70–90%) was positive for p24 and p15. c, Cord blood cells removed 18 days after transfection with pCH-Igpt (HTLV-I clone) were not labelled by these antibodies. f, Electron micrographs of cord blood cell cultures transfected with pHXB-2D show the presence of extracellular HTLV-III virus particles in cultures 18 days after protoplast fusion ($\times 45,000$).

able to produce fully infectious virus which is then transmitted within the culture.

No HTLV-III sequences were detected 31 days after transfection (Fig. 3b); at this point the culture may have contained only cells which failed to be infected by HTLV-III. This result is again consistent with the transfected DNA exerting a cytopathic effect on T cells. The finding that, at any stage, only a minor population of the transfected cells are apparently infected by the virus (<15% express viral proteins) suggests that the cytopathic effects may not result solely from direct viral infection and that secreted factors and/or other cell-to-cell interactions may play a part in the cytopathic phenomenon. To confirm this, we are presently using an *in situ* RNA hybridization technique to accurately assess the proportion of cells expressing viral messenger RNA transcripts (as this may be higher than the proportion expressing p15 or p24) (A.G.F. *et al.*, in preparation).

We have described the construction of a molecular clone of HTLV-III and its introduction into normal T cells, and demonstrated that T cells transfected with this clone contain unintegrated HTLV-III proviral sequences, express viral antigen and produce mature virus particles. Furthermore, we provide evidence that the virus produced from the HTLV-III genome is infectious and has marked cytopathic effects *in vitro*. Thus, HTLV-III is itself pathogenic for normal T cells. These results exclude the possibility that a minor component of the virus

complex is solely responsible for, or acts as a co-factor in, the cytopathic effects. The availability of this clone will allow us to elucidate the biological functions of the HTLV-III genome and, in particular, to localize the regions governing the cytopathic properties of the virus. In addition, as transfection allows the introduction of viral DNA into cells not normally infected by HTLV-III, we can use this system to investigate whether the tropism displayed by HTLV-III is determined exclusively at the level of the receptor^{26,27} or also at an intracellular level.

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Antibodies against metal chelates

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Because monoclonal antibodies can recognize and bind to specific groups of atoms such as tumour antigens, they have promise for use *in vivo* as carriers of radionuclides, drugs or other appended molecules for diagnosis and treatment of disease^{1–3}. Attachment of metal ions to antibodies by means of bifunctional chelating agents can add the diverse nuclear, physical and chemical properties of the metallic elements to these specific binding proteins (ref. 4 and refs therein). With the ultimate aim of engineering

probe-binding properties into the antibodies themselves, we have now prepared monoclonal antibodies against the EDTA chelate of indium. These antibodies show a remarkable preference for indium chelates; changing to another metal such as scandium or gallium can decrease the antibody-binding constant by more than three orders of magnitude. These antibodies also introduce a new degree of control over the biological distributions of chelated radionuclides, markedly altering their uptake in tumours and normal organs.

Figure 1 shows the approximately 70 metallic elements that form stable EDTA chelates^{5,6}. These may possess properties such as radioactivity, luminescence or paramagnetism, leading to new molecular labels for use in nuclear medicine, immunoassay or magnetic resonance imaging^{2,3,7-10}. In solution, many of these chelates exist in dynamic equilibrium between several molecular configurations^{11,12}, but they can be crystallized as unique structures. Figure 2 shows a structure of iron(III)-EDTA; the EDTA chelates of indium(III), cadmium(II), cobalt(II) and manganese(II) crystallize in similar configurations¹³⁻¹⁷. Many other metal-EDTAs have different structures¹⁸⁻²³, thus, it is interesting to investigate whether an antibody against one metal-chelate can bind others in which the metal (or the chelator) is different.

To make antibodies against indium-EDTA, an antigen was prepared by conjugating L-SCN-C₆H₄-CH₂-EDTA to keyhole limpet haemocyanin using the methods of Meares *et al.*²⁴. After addition of indium(III), there was ~0.1 mg of attached chelate per mg of protein. To prepare antibody-producing hybridoma cell lines, spleen cells from BALB/c mice (multiply immunized with the antigen) were fused with a variant of the P3.653 myeloma cell line using the technique of Gerhard²⁵. The resulting hybridomas were screened by radioimmunoassay²⁶ for their ability to bind ¹¹¹In-L-NH₂-C₆H₄-CH₂-EDTA or ¹²⁵I-labelled bovine serum albumin bearing nonradioactive indium-EDTA groups; of 3,476 clones sampled, 102 produced antibodies possessing measurable affinity for the chelate. Based on their high titres and affinities, two IgG1 antibodies (from clones CHA255 and CHB235) were selected for further study. These monoclonal antibodies were purified from mouse ascites fluid

TABLE OF THE ELEMENTS																		VIIIA				
IA																	IIIA	IVA	VA	VIA	VIIA	
IIA	Be																					
	Mg	IIIB	IVB	VB	VIB	VII B	VIII			IB	II B	Al										
	Ca	<u>Sc</u>	Ti	V	Cr	<u>Mn</u>	<u>Fe</u>	<u>Co</u>	Ni	<u>Cu</u>	<u>Zn</u>	<u>Ga</u>										
	Sr	Y	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	<u>Cd</u>	<u>In</u>	Sn									
	Ba	La	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi								
	Ra	Ac																				
Lanthanide Series		Ce	Pr	Nd	Pm	Sm	Eu	Gd	<u>Tb</u>	Dy	Ho	Er	Tm	<u>Yb</u>	Lu							
Actinide Series		Th	Pa	U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No								

Fig. 1 Metals that form stable EDTA chelates^{5,6}. Elements studied here are shown in bold face. A single underline indicates that the affinity of both antibodies for the metal-L-benzyl-EDTA chelate is 10–100 times less than for indium-L-benzyl-EDTA; a double underline 100–1,000 times less; a triple underline 1,000–10,000 times less.

by ion-exchange chromatography on DEAE-cellulose²⁷. Purity was routinely checked by SDS-polyacrylamide gel electrophoresis²⁸; for each purified sample, only one light-chain band and one heavy-chain band could be detected.

Binding constants for indium chelates were determined in triplicate by equilibrium dialysis and Scatchard analysis of a series of antibody-chelate solutions²⁹; as expected for IgG1, each antibody had 2 (± 0.2) binding sites for chelate. Indium-EDTA labelled with ¹¹¹In or ¹⁴C was used in parallel experiments (with similar results), confirming that each antibody binds the chelator as well as the metal. The binding constants for metal chelates other than In-L-benzyl-EDTA were determined by equilibrium dialysis in competition with ¹¹¹In-L-benzyl-EDTA; this procedure was validated by independent determinations of the binding constants of indium-[¹⁴C]EDTA and ⁵⁷Co-L-benzyl-EDTA, and also by isotope dilution using non-radioactive In-L-benzyl-EDTA. ¹¹¹In-L-benzyl-EDTA did not bind detectably (<0.1%) to normal whole mouse IgG or to mouse serum.

Metal chelates selected for testing (Fig. 1) included trivalent ions that are similar to indium (Sc, Ga, Fe), and divalent ions whose EDTA chelates can have structures similar to that of indium-EDTA (Mn, Co, Cd). Inspection of Table 1a shows that changing the metal in the chelate can decrease the antibody-binding constant K_b , by more than three orders of magnitude. The metals whose chelates bind best are indium(III), iron(III) and cadmium(II); the available crystal structures for their chelates are all similar to that in Fig. 2¹³⁻¹⁷. Table 1 also shows that the K_b values are not well correlated with either ionic radii or metal-chelate stability constants.

Each antibody is much like the other with regard to selectivity for metals (Fig. 1); however, the antibodies differ markedly in their sensitivity to changes in the chelator. Table 1b shows that CHB235 is almost unaffected by whether the side chain is in the D- or L-configuration (see Fig. 2), or whether there is a side chain at all. CHA255 is rather sensitive to the presence of the L-benzyl side chain.

Because metal ions have affinity for amino-acid side chains bearing sulphur, nitrogen or oxygen^{5,6}, antibodies are fundamentally well suited to binding metal ions or metal chelates. Some cancer patients have been found to produce myeloma proteins (antibodies) that bind copper or calcium ions^{30,31}, and certain heavy metals such as platinum can be immunogenic³²⁻³⁴. The methods used here can be applied to prepare antibodies against a variety of metal chelates. It is immediately clear that particular metal chelates—and therefore particular metal ions—can be selectively bound, analysed and manipulated by immunochemical techniques^{2,3,35}. This novel connection of immunochemistry to inorganic chemistry may find practical use; for example, in trace element analysis by immunoassay.

Table 1 Relative binding of metal chelates to monoclonal antibodies against an indium-EDTA analogue

a. Chelates of L-benzyl-EDTA with different metal ions				
Metals	r_{ion} (Å)	$\log K_s$ (EDTA)	K_b (CHA255)	K_b (CHB235)
Sc(III)	0.81	23.1	$3.0 \times 10^6 (\pm 13\%)$	$5 \times 10^4 (\pm 39\%)$
Fe(III)	0.64	25.1	$1.8 \times 10^8 (\pm 13\%)$	$2.4 \times 10^6 (\pm 18\%)$
Ga(III)	0.62	20.3	$5 \times 10^7 (\pm 45\%)$	$5.2 \times 10^5 (\pm 16\%)$
In(III)	0.81	25.0	$4.0 \times 10^9 (\pm 6.9\%)$	$1.1 \times 10^8 (\pm 7.9\%)$
Tb(III)	1.00	17.9	$1.2 \times 10^6 (\pm 12\%)$	$2.7 \times 10^4 (\pm 15\%)$
Yb(III)	0.94	19.5	$1.1 \times 10^6 (\pm 20\%)$	$2 \times 10^4 (\pm 90\%)$
Mn(II)	0.80	13.9	$2.8 \times 10^6 (\pm 9.1\%)$	$2.8 \times 10^4 (\pm 24\%)$
Co(II)	0.74	16.3	$2 \times 10^6 (\pm 37\%)$	$9.4 \times 10^5 (\pm 22\%)$
Co(III)	0.63	41.4	$8 \times 10^7 (\pm 60\%)$	$< 1 \times 10^4$
Cu(II)	0.69	18.8	$1.7 \times 10^6 (\pm 12\%)$	$8.1 \times 10^4 (\pm 11\%)$
Zn(II)	0.74	16.5	$1.4 \times 10^6 (\pm 14\%)$	$3.5 \times 10^4 (\pm 27\%)$
Cd(II)	0.97	16.5	$1.5 \times 10^7 (\pm 8.0\%)$	$1.6 \times 10^5 (\pm 8.1\%)$
b. Indium chelates with different chelators				
Chelators			K_b (CHA255)	K_b (CHB235)
L-Benzyl-EDTA			$4.0 \times 10^9 (\pm 6.9\%)$	$1.1 \times 10^8 (\pm 7.9\%)$
D-Benzyl-EDTA			$6 \times 10^7 (\pm 40\%)$	$3.6 \times 10^7 (\pm 9.3\%)$
EDTA			$1.7 \times 10^6 (\pm 8.3\%)$	$1.3 \times 10^6 (\pm 13\%)$
HED3A			$4.2 \times 10^7 (\pm 12\%)$	$4.0 \times 10^7 (\pm 14\%)$

a. Column 1, the metals whose L-benzyl-EDTA chelates (see Fig. 2) were tested against the indium chelate. Column 2, the ionic radius of each metal⁴⁰. Column 3, the logarithm of the stability constant^{5,6} for each metal-EDTA chelate at 25 °C. Columns 4 and 5, the antibody-binding constants of each metal-L-benzyl-EDTA chelate for the two different monoclonal antibodies studied (CHA255 and CHB235), in 0.05 M 2-hydroxyethyl piperazine ethane sulphonate, 0.1 M NaCl, 0.1% Na₂S₂O₄, 0.1% bovine serum albumin, pH 7.37 °C. Relative standard deviations are given in parentheses. For 'metal-free' Na⁺ L-benzyl-EDTA, K_b (CHA255) is $< 10^5$; in this case it is difficult to measure K_b accurately, as trace metal contaminants bind to the chelator. b. Column 1, the different chelators used to make indium chelates (see Fig. 2).

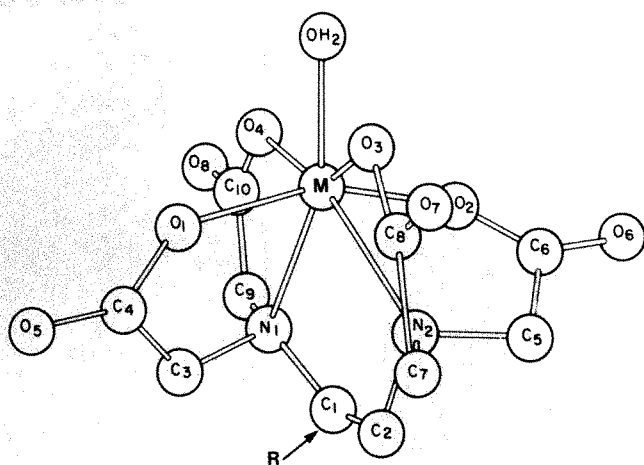


Fig. 2 Crystal structure of the Fe(III)-EDTA chelate, which is similar to those of In(III), Cd(II), Mn(II) and Co(II)¹³⁻¹⁷. EDTA supplies two nitrogen (N1, N2) and four oxygen (O1-O4) ligands to the metal (M), and a water molecule (OH₂) is also coordinated to the metal. For many of the experiments described here, a benzyl side chain (R = C₆H₅CH₂—) was attached to carbon-1, as indicated by the arrow (both the D- and L-configurations were studied). The chelator HED3A has an alcohol in place of one carboxylate group (for example, by replacement of oxygen-5 with two hydrogens). Drawing adapted from Hoard *et al.*¹⁴.

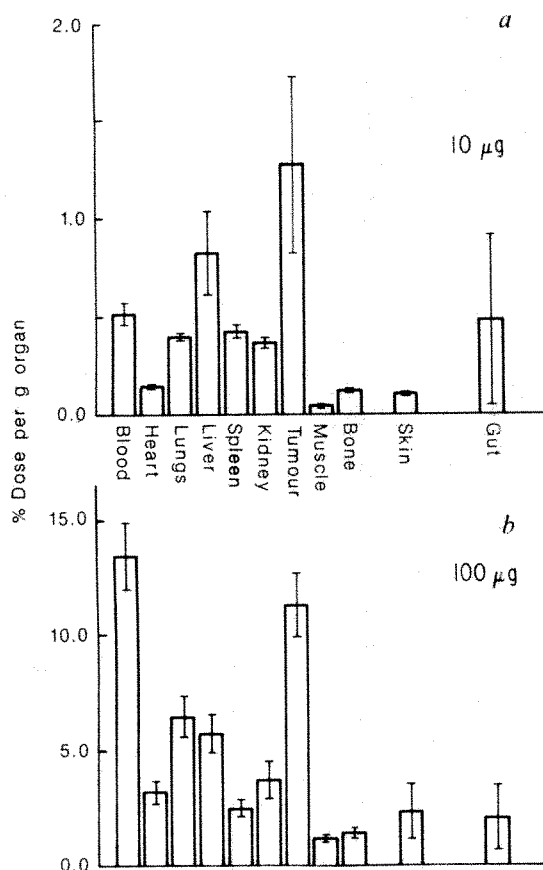


Fig. 3 Comparison of the distributions of radioactivity in BALB/c mice bearing KHJJ tumours³⁶, 24 h after injection of either 10 µg CHA255 anti-chelate antibody plus a trace of ¹¹¹In-L-benzyl-EDTA (a) or 100 µg CHA255 plus ¹¹¹In-L-benzyl-EDTA (b). Error bars represent 1 s.d. Note that the range of concentrations in b is 8.5 times that in a. Similar results were obtained with the CHB235 antibody, but larger amounts were required. When ¹¹¹In-L-benzyl-EDTA was injected without anti-chelate antibody, the concentrations of radioactivity were all less than 0.05% per g; this is insignificant on the scale of a.

These antibodies are also capable of altering the biological properties of metal chelates. For example, consider their effect on the behaviour of ¹¹¹In-L-benzyl-EDTA in BALB/c mice. Intravenous injection³⁶ of a trace of the radiochelate together with 10 µg of CHA255 per mouse increases the biological half-life of the chelate from 40 ± 15 min to 6 ± 1 h; when the amount of antibody is increased to 100 µg, the half-life increases to 54 ± 15 h. Nonspecific antibody, or quantities of specific antibody smaller than 1 µg per mouse, have no measurable effect on the biological behaviour of the indium chelate.

These 'reversibly radiolabelled' antibodies behave in a way that is qualitatively different from covalently radiolabelled antibodies. Figure 3 shows tumour and organ concentrations of radioactivity 24 h after injection of ¹¹¹In-L-benzyl-EDTA and anti-chelate antibody in mice bearing KHJJ tumours. By varying the antibody concentration *in vivo*, it is possible to enhance the uptake of radioactivity in the tumour relative to the rest of the body (Fig. 3a). All the radioactivity concentrations are strikingly increased in the presence of the antibody, with the tumour concentration rising from <0.05% per g up to 11.3% per g (Fig. 3b). Clearly, anti-chelate antibodies can introduce a new degree of control over the biological distribution of chelated radionuclides and other chelates used in medical diagnosis and therapy.

As the antibodies have no known affinity for the tumour, they are presumed to accumulate passively³⁷. Extension of the present work to prepare monoclonal antibodies with dual antigen specificity^{38,39}, which could simultaneously bind a metal chelate and a physiological antigen, may provide further improvements.

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Active γ -carboxylated human factor IX expressed using recombinant DNA techniques

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Factor IX (Christmas factor), a vitamin K-dependent plasma protein made in the liver, functions in the middle phase of the intrinsic pathway of blood coagulation¹. A functional deficiency of factor IX underlies haemophilia B, a chromosome X-linked recessive disease for which the major therapeutic approach is replacement treatment using factor IX concentrates. The cloning and characterization of the gene for human factor IX²⁻⁴ would mean that human factor IX could be produced in greater yield and purity through using recombinant DNA techniques⁵. We have now used a human factor IX cDNA clone⁴, inserted into a vaccinia virus-derived vector, to infect human hepatoma cells which normally produce no factor IX⁶, and mouse fibroblasts. Fully active factor IX was produced by the hepatoma cells, whereas the fibroblasts produced a protein less active than natural factor IX, even in the presence of high levels of vitamin K. Human factor IX is extensively post-translationally modified, and thus represents probably the most complex protein produced in active form by recombinant DNA techniques to date. Our study also illustrates the potential of vaccinia virus-based vectors for expressing significant amounts of complex, clinically useful proteins in eukaryotic cells, in addition to its already demonstrated usefulness for producing live recombinant vaccines⁷⁻⁹.

Factor IX is a protein of extraordinary complexity. Several sialic carbohydrates are linked to the protein chain and experiments have shown that these groups may be important for the activity of the molecule¹⁰. The first 12 glutamic acid residues of the mature enzyme are converted to γ -carboxyglutamic acids in a vitamin K-dependent process. These modifications are essential for the activation of factor IX. In addition, one aspartic acid residue (position 64) is hydroxylated¹¹⁻¹³, and may also be important for activation of the molecule^{11,12,14}.

Activation of factor IX from its zymogen form occurs through the action of activated factor XI. It involves two specific proteolytic cleavages, and releases an activation peptide, and the active proteolytic enzyme, factor IXa, whose two chains are held together by disulphide bridges. In addition, analysis of the factor IX gene sequence shows that the primary translation product of factor IX messenger RNA is a pre-pro form^{3,4}, though the zymogen precursor has not yet been characterized.

In a search for cells which can produce biologically active factor IX, we attempted to express the protein in several different cell lines. We first looked at a human hepatoma cell line, HepG2 (ref. 6), which synthesizes many different plasma proteins,

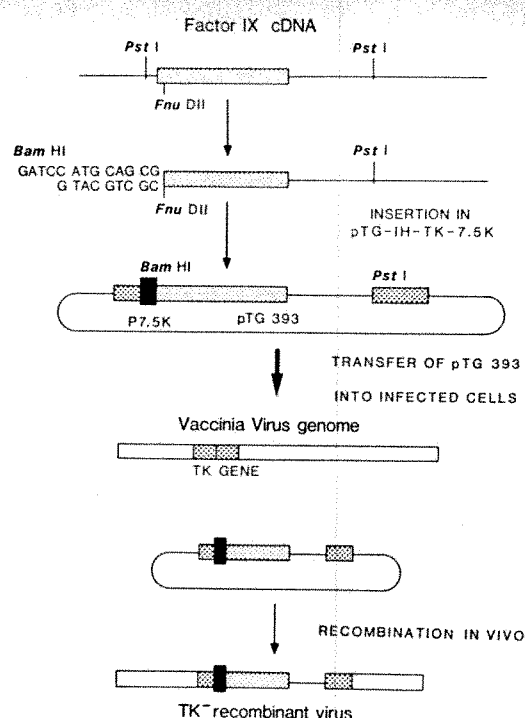


Fig. 1 Insertion of the factor IX cDNA coding sequence into vaccinia virus. The *Fnu*DII/*Pst*I fragment was isolated from the factor IX cDNA clone⁴, and the coding sequence at the 5' end together with a *Bam*HI site cohesive end were restored using a synthetic DNA linker. This fragment was then transferred to the plasmid pTGIH-tk-P7.5K (ref. 9) which contains the vaccinia virus *tk* gene insertionally inactivated by the presence of the early 7.5K promoter of vaccinia virus. The resulting plasmid, pTG393, contains the factor IX cDNA sequence immediately adjacent to the 7.5K promoter, and flanked on either side by parts of the vaccinia *tk* gene. These flanking regions allowed the *in vivo* recombination of the factor IX sequence of pTG393, together with the 7.5K promoter, into the N7 strain of vaccinia virus (derived from the Copenhagen strain) using procedures already described⁹. The fine dotted regions represent the factor IX cDNA sequence, the larger dotted regions the vaccinia *tk* gene, and the black area the 7.5K promoter.

including factor X and protein C, which are closely related to factor IX and undergo many of the same modifications. However, no factor IX synthesis was found in these cells⁶, nor have we been able to detect significant levels of factor IX mRNA in the cells (unpublished observations).

To express factor IX in HepG2 cells, we used vaccinia virus as a vector. The strategy for insertion of a gene into the vaccinia genome has been described previously⁷⁻⁹ and is based on a homologous recombination between the thymidine kinase (TK) gene in the viral genome and the plasmid bearing the gene to be expressed, generating a TK⁻ recombinant virus (Fig. 1). One such TK⁻ virus was analysed by Southern blotting to verify that the pattern of restriction fragments of the cDNA inserted in the vaccinia genome was the same as in the starting plasmid (Fig. 2).

To establish whether the inserted factor IX gene was expressed during vaccinia infection, HepG2 cells infected for 15 h were labelled for 5 h with ³⁵S-methionine. Both cell extracts and culture medium were immunoprecipitated with IgGs prepared from rabbit anti-human factor IX antiserum. Two relatively diffuse bands, of apparent relative molecular masses (*M*_s) 79,000 (79K) and 71K, were specifically immunoprecipitated from the medium of HepG2 cells infected with the factor IX recombinant vaccinia virus (Fig. 3A, lane 2) but were absent from cells infected with wild-type virus (lane 1) or from media precipitated with non-immune serum (lanes 5-8). The smaller of the specifically immunoprecipitated bands has an apparent

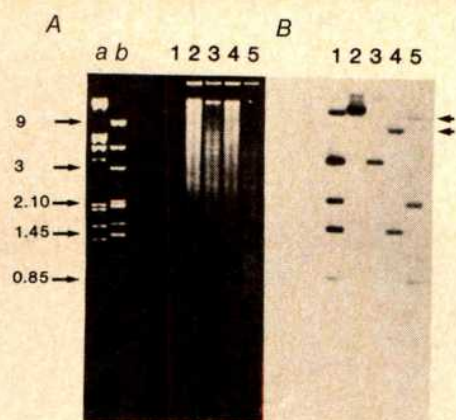


Fig. 2 Verification of the insertion of the factor IX cDNA sequence into vaccinia virus. **A**, Ethidium bromide-stained gel before transfer. Lane *a*, M_r standards from a digest of λ DNA with *EcoRI* and *HindIII*; lane *b*, a mixture of different digests of the plasmid, pTG393, carrying the factor IX sequence within the vaccinia *tk* gene. A different digest, with *PstI* alone, *PstI* + *HindIII*, *PstI* + *AvaI* and *PstI* + *AvaII* were mixed before electrophoresis. The fragments which should contain factor IX sequences are marked by arrows, and have sizes of 9.0, 3.0, 2.11, 1.45 and 0.85 kilobases, respectively. Lane 1, mixture of pTG393 digests as for lane *b*, but with 10 ng of DNA instead of 1.0 μ g; lanes 2–5, DNA of infected cells digested with *PstI* (lane 2), *PstI* + *HindIII* (3), *PstI* + *AvaI* (4), *PstI* + *AvaII* (5). **B**, Autoradiogram of nitrocellulose filter hybridized with ^{32} P-labelled factor IX probe. Lanes 1–5 correspond to the same lanes as for **A**. The two largest bands in lanes 4 and 5 (arrows on right) correspond to end fragments of the factor IX portion of pTG393 flanked by vaccinia sequence, and so do not correspond in size to the hybridizing fragments of pTG393 alone (lane 1).

Methods. A 75-cm² flask of confluent chick embryo primary fibroblast cells was infected with 0.5 plaque-forming units per cell of recombinant vaccinia virus. After 2 days, cells were collected, lysed and DNA was prepared by standard procedures. The DNA was digested with various restriction enzymes, electrophoresed on a 1% agarose gel and blotted onto nitrocellulose. The filter was hybridized with ^{32} P-labelled factor cDNA probe.

size consistent with that of natural factor IX; the other may represent a precursor form of factor IX, or perhaps a molecule containing a different form of glycosylation.

The analysis of supernatants from HepG2 cells infected and labelled in the presence of tunicamycin, an inhibitor of *N*-glycosylation, revealed several specific bands of greater mobility from cells infected with the recombinant vaccinia virus (Fig. 3A, lane 3). Moreover, the ability of unlabelled factor IX to compete with the medium polypeptides (Fig. 3B, lanes 1, 3) provided additional evidence that the immunoprecipitated polypeptides were factor IX molecules.

To characterize further recombinant factor IX produced by the vaccinia vector, and to determine whether the product exhibited biological activity, medium from infected cells was fractionated using barium citrate¹⁵. This fractionation is based on the ability of γ -carboxylated proteins to adsorb to the insoluble salt of barium citrate¹⁵. Fully γ -carboxylated molecules co-precipitate with the salt, while non-carboxylated molecules stay in solution. The properties of a partially modified protein are not known in the case of factor IX, but studies with prothrombin, another γ -carboxylated protein, have shown that the extent of precipitation is related to the number of γ -carboxyglutamic acid residues present¹⁵. We used a commercially available enzyme-linked immunosorbent assay to quantitate factor IX antigen through the fractionation procedure, and the activity of barium citrate-adsorbed fractions was measured as their ability to restore coagulability to plasma congenitally deficient in factor IX¹⁶. Both factor IX antigen and activity are expressed in arbitrary units; 1 unit corresponds to the quantity of factor IX in 1 ml of normal human pooled plasma ($\sim 5 \mu$ g).

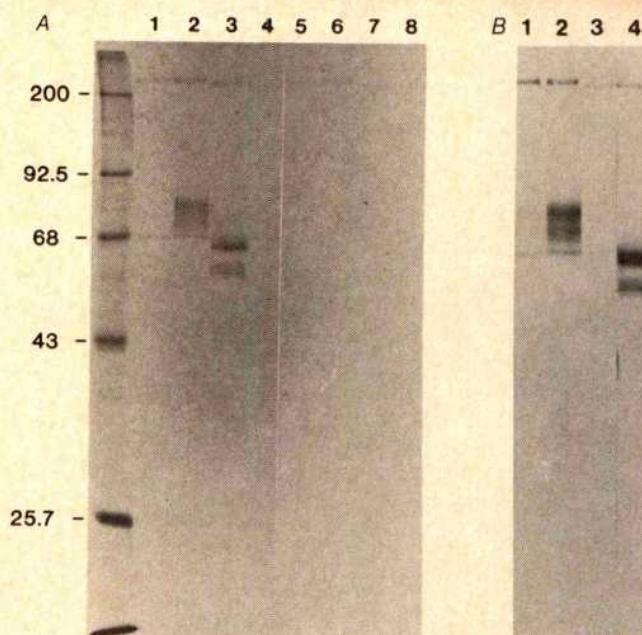


Fig. 3 Immunoprecipitation of recombinant human factor IX. **A**, Immunoprecipitates with labelled cell media. Lane 1, medium from cells infected with wild-type virus; lane 2, medium of cells infected with recombinant virus; lane 3, as for lane 2, but cells grown in tunicamycin; lane 4, as for lane 1, but cells grown in the presence of tunicamycin; lanes 5–8, as for lanes 1–4, but non-immune IgG used instead of anti-factor IX IgG. Numbers on the left represent M_r markers ($\times 10^{-3}$). **B**, Immunoprecipitation with native factor IX. Competitions were performed by adding 10 μ g of natural human factor IX to cell media or extracts before IgG addition. Lane 1, medium from cells precipitated in the presence of competitor; lane 2, as for lane 1, but without competitor; lane 3, medium from tunicamycin-treated cells precipitated in the presence of competitor; lane 4, as for lane 3, but without competitor.

Methods. Confluent hepatoma cells were infected with either recombinant or wild-type vaccinia virus, either in the presence or absence of 1μ g ml⁻¹ tunicamycin. After 24 h of infection, the medium was removed and replaced by a synthetic medium lacking cold methionine. ^{35} S-methionine (specific activity ≥ 800 Ci mmol⁻¹) was added at 50μ Ci ml⁻¹, and labelling was carried out for 5 h to obtain labelled supernatants. For cells pretreated with tunicamycin, the compound was also present at 1μ g ml⁻¹ in the labelling medium. Cells and media were collected, the cells were lysed in 1% Triton X-100 and aliquots of the medium (10^5 c.p.m. of trichloroacetic acid-precipitable radioactivity) were subjected to immunoprecipitation¹⁷ with rabbit anti-human factor IX IgG or non-immune rabbit IgG. The immunoprecipitates were analysed by 7.5% polyacrylamide gel electrophoresis¹⁸, followed by fluorography and autoradiography.

We infected both HepG2 and the mouse fibroblast LM-TK⁻ cell lines with either the wild type or the recombinant vaccinia virus and analysed the medium at several times after absorption of the virus (Table 1a). Although the factor IX complementary DNA is under the control of the early 7.5K promoter, in the recombinant vaccinia virus construct, we obtained maximal expression of both antigen and activity after ~ 22 h of infection. In fact, the 7.5K promoter is known to be expressed during the entire viral cycle, so the high levels of late expression probably appear after the replication of the viral genome. In medium from cells infected with wild-type vaccinia virus, no significant levels of either factor IX antigen or activity were observed. It is also significant that although there is a good correlation between antigen and activity in the barium citrate-adsorbed fraction for HepG2 cells, this is not the case for the LM-TK⁻ cells (see below). The differences in absolute levels of factor IX produced probably do not result from differences in expression in the two different cell lines, but rather correlate with the

Table 1 Time course of factor IX production (a) and effect of vitamin K (b)

a	Cell line	HepG2						LM-TK ⁻			
		w.t.			rec.			rec.		w.t.	
Virus											
Time after infection (h)		4	8	22	4	8	22	6	16	22	22
Units factor IX non-adsorbed to barium (antigen)		0.01≤	0.01≤	0.01≤	0.03	0.04	0.01	0.09	0.05	0.14	0.01≤
Units of factor IX adsorbed to barium (antigen)		0.01≤	0.01≤	0.01≤	0.01	0.03	0.71	0.02	0.04	0.40	0.01≤
Units of factor IX adsorbed to barium (activity)		0.01	0.01	0.01	0.03	0.04	0.57	0.03	0.04	0.12	0.04
b											
Virus		w.t.		rec.	w.t.		rec.	rec.		w.t.	rec.
Vitamin K added to medium		-		-	+		+	-		+	+
Total factor IX in medium (antigen)		≤0.1		2.1	≤0.1		1.9	ND		ND	ND
Factor IX non-adsorbed to barium (antigen)		0.01		0.43	0.01		0.04	1.06		0.01	0.18
Factor IX adsorbed to barium (antigen)		≤0.01		0.49	≤0.1		0.77	0.16		≤0.01	0.66
Factor IX adsorbed to barium (activity)		0.01		0.18	0.02		0.62	0.05		0.02	0.20

a, HepG2 and LM-TK⁻ cells were grown to confluence on 75-cm² plates (~2 and 1 × 10⁷ cells, respectively), then infected with the recombinant factor IX-containing vaccinia virus. These infections were performed in modified Eagle's (MEM) medium supplemented with 10% fetal calf serum (FCS) and 50 µg ml⁻¹ vitamin K. The medium was removed at various times after infection, fractionated using barium citrate as described in the text, and factor IX antigen and activity were measured. b, HepG2 cells were passaged five times in MEM supplemented with 2% serum substitute Ultrosor G (IBF) to lower endogenous vitamin K levels of the cells. Then confluent monolayers in the same medium were infected with the recombinant vaccinia virus, either in the presence or absence of 50 µg ml⁻¹ vitamin K. For the LM-TK⁻ cells, confluent monolayers grown in MEM + 10% FCS were infected in the presence or absence of added vitamin K (50 µg ml⁻¹). Cell media were fractionated, and factor IX antigen and activity were measured as above. For these experiments vitamin K was dissolved in ethanol (50 mg ml⁻¹), then added to the medium. The results are expressed in units, 1 unit corresponding to the quantity of factor IX in 1 ml of normal human pooled plasma. w.t., Wild-type N7 Copenhagen vaccinia virus; rec., recombinant virus; ND, not determined.

number of cells used. Subsequent experiments were performed using medium from cells infected for ~24 h.

As γ-carboxylation of the glutamic acid residues of factor IX is vitamin K-dependent, we investigated the effect of vitamin K on the production of recombinant factor IX (Table 1b). Vitamin K had no effect on the total production of factor IX by HepG2 cells infected with vaccinia, in contrast to the vitamin K-dependent stimulation of prothrombin synthesis reported for this cell line⁶. However, the vitamin did have a marked effect on the distribution of factor IX between the two fractions produced by barium citrate fractionation. When vitamin K was not added, a large part of the synthesized factor IX did not adsorb to barium citrate, but when it was present most of the factor IX bound to the salt.

The factor IX molecules produced by infected HepG2 cells were fully active if vitamin K was present in excess in the medium, but when it was absent the specific activity of factor IX adsorbed to barium citrate was halved. We propose that partially carboxylated proteins can bind to the barium salt, but exhibit a lower biological activity. Similar results were obtained with LM-TK⁻-infected cells, but a striking difference was seen between the factor IX produced by these cells and that from HepG2 cells. Even with an excess of vitamin K in the medium, the molecules produced by infected LM-TK⁻ cells and adsorbed to barium citrate appeared to be three times less active than natural factor IX. As we also found a significant amount of factor IX antigen in the non-adsorbed fraction, we consider that the γ-carboxylation must be less efficient in this cell line. This would explain why the factor produced by LM-TK⁻ cells, even in the presence of vitamin K, was not fully active.

We have demonstrated here the feasibility of producing biologically active factor IX in mammalian cells. The concentration

of factor IX activity in supernatants of infected cells is less than that present in pooled human plasma, but this represents a first step towards the production of a highly purified recombinant protein that could be used to treat haemophilia B.

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Expression of active human factor IX in transfected cells

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Factor IX is the precursor of a serine protease that functions in the intrinsic blood clotting pathway¹. Deficiencies in this plasma glycoprotein result in haemophilia B (or Christmas disease) and occur in about 1 in 30,000 males². Patients are currently treated with fresh frozen plasma or prothrombin complex concentrates prepared from pooled plasma from normal individuals. There are several problems with this method of treatment, including the probable exposure of the patients to contaminants such as the viral agents responsible for hepatitis and AIDS (acquired immune deficiency syndrome). As a first step towards an alternative source of pure human factor IX, we report here on the use of recombinant DNA techniques to produce biologically active factor IX in cultured mammalian cells. Stable cell lines were produced by co-transfecting a baby hamster kidney (BHK) cell line with a plasmid containing a gene for factor IX and a plasmid containing a selectable marker. Protein secreted by these cell lines reduces the clotting time of plasma from factor IX-deficient patients. We present additional evidence that this protein is authentic human factor IX.

We report here the expression of an active vitamin K-dependent protein using recombinant DNA techniques. Factor IX is one of six identified plasma proteins whose biosynthesis is dependent on this vitamin^{1,3}. Vitamin K is a cofactor of the microsomal enzymes that perform post-translational modifications to produce the γ -carboxyglutamic acid (gla) residues characteristic of these proteins. The gla residues, which bind Ca^{2+} and allow the protein to interact with phospholipid, are necessary for biological activity⁴.

Human factor IX circulates in the blood as a single-chain glycoprotein of M_r 57,000 (ref. 1). During blood coagulation, it is converted to an active serine protease (factor IXa) after cleavage by factor XIa. Factor IXa, which consists of a light chain and a heavy chain (M_r 16,000 and 29,000, respectively) held together by a disulphide bond(s), then converts factor X to factor Xa in the presence of factor VIIIa, phospholipid and Ca^{2+} (ref. 1).

To express factor IX in cultured cells, we used a complementary DNA⁵ containing 138 base pairs (bp) that encode the 46-amino-acid leader, 1,248 bp that encode the 415-amino-acid mature protein and 48 bp of 3' noncoding DNA sequence. As described in Fig. 1 legend, this cDNA was inserted into a plasmid containing the adenovirus-2 major late promoter, a set of RNA splice sites and the simian virus 40 (SV40) polyadenylation signal. BHK cells were co-transfected with the factor IX plasmid (FIX/pD2) and pKOneo, a plasmid containing the gene for resistance to the antibiotic G418 (ref. 6). G418-resistant colonies were isolated and screened for the presence of factor IX activity secreted into the media; ~50% of the resistant colonies analysed secreted detectable factor IX activity. In subsequent analyses of a representative clone (IX-F), the supernatant media and the cell pellets of clone IX-F were assayed for the amounts of biologically active factor IX and factor IX polypeptide. The assay for biological activity was based on the ability of the sample to reduce the blood clotting time of plasma from factor IX-deficient patients⁷ and the amount of factor IX polypeptide was determined by an enzyme-linked immunosorbent assay (ELISA) using an affinity-purified polyclonal rabbit antibody to factor IX. The cells were grown for different lengths of time, and the media and cells collected for analysis. Most of the factor

Table 1 Production of factor IX by cell line IX-F

Day	Cell no. $\times 10^4$ per ml of medium	Factor IX activity in medium (ng ml ⁻¹)	Factor IX polypeptide (ng ml ⁻¹)		% Active protein in medium
			Supernatant medium	Cell pellet	
1	1.86	ND	ND	ND	
	1.44				
2	2.73	27	57	20	55
	2.58	24	45	20	
3	9.99	72	150	60	60
	9.39	84	120	60	
4	17.58	198	475	160	55
	12.00	150	225	140	
5	48.90	408	875	250	46
	52.80	438	1,000	260	
6	66.00	648	1,500	220	46
	72.00	678	1,375	290	

Cells from clone IX-F were plated at 1.5×10^5 cells per 10-cm dish in Dulbecco's modified Eagle's medium containing 10% fetal calf serum and $1 \mu\text{g ml}^{-1}$ vitamin K (phytonadione, Merck). At the indicated time, the medium was aspirated, centrifuged at 2,000 r.p.m. for 10 min and frozen at -80°C . The cells were trypsinized, washed twice with Dulbecco's medium and counted. The cell pellet was frozen at -80°C . For the protein assays, the supernatant samples were thawed and assayed for biological activity by a one-stage clotting assay. This assay is based on the ability of the sample to decrease the prolonged activated partial thromboplastin time of congenital factor IX-deficient plasma. Normal control plasma (0.1 ml) (George King Biomedical Co.) or supernatant medium (0.1 ml) was incubated with factor IX-deficient plasma (0.1 ml) (George King Biomedical Co.) and cephaline-kaolin mixture⁷ (0.1 ml) for 12 min at 37°C . Then 40 mM CaCl_2 (0.1 ml) was added and the clotting time recorded. The factor IX activity was expressed as a percentage of the activity present in normal plasma and then converted to ng ml⁻¹ using a standard value of $3 \mu\text{g ml}^{-1}$ of factor IX in normal plasma. The cell pellets were extracted by freeze-thawing three times in 2 ml phosphate-buffered saline (PBS) and then centrifuged for 5 min in a microfuge. The pellet was re-extracted in 1 ml PBS, 0.25% Triton X-100. The samples were diluted 1:5 for ELISA. The amount of factor IX polypeptide present in the samples was measured by ELISA as follows. Affinity-purified rabbit polyclonal antibody against human factor IX ($200 \mu\text{l}$ of $1 \mu\text{g ml}^{-1}$ in 0.1 M Na_2CO_3 , pH 9.6) was incubated in each well of 96-well microtitre plates overnight at 4°C or for 2 h at 37°C . The wells were washed three times with PBS, 0.05% Tween 20 and then incubated with $220 \mu\text{l}$ of 1% bovine serum albumin (BSA), 0.05% Tween 20 in PBS, pH 7.2 overnight at 4°C or for 2 h at 37°C . The plates were rinsed several times in distilled water, air-dried and stored at 4°C . To assay samples containing factor IX, $200 \mu\text{l}$ of each sample was incubated for 1 h at room temperature in the antibody-coated wells. The wells were rinsed four times with 0.05% Tween 20 in PBS and then incubated for 1 h at room temperature with affinity-purified rabbit polyclonal anti-factor IX conjugated to alkaline phosphatase in PBS containing 1% BSA and 0.05% Tween 20. The per cent active protein was calculated by dividing the amount of factor IX activity in the medium by the amount of factor IX polypeptide in the medium. The same analyses were performed on spent media and cell pellets from control BHK cells and no factor IX was detected. To produce clone IX-F, the plasmid FIX/pD2 was introduced into BHK cells by calcium phosphate-mediated transfection¹¹. To transfect one 100-mm dish of 20% confluent cells, a total of $20 \mu\text{g}$ DNA was used: $5 \mu\text{g}$ of FIX/pD2, $5 \mu\text{g}$ pKOneo and $10 \mu\text{g}$ salmon sperm DNA. Twenty-four hours after transfection, selective medium (0.5 mg ml^{-1} , Gibco G418) was added. Resistant colonies were picked after 10–13 days. ND, not detectable.

IX polypeptide (70–80%) was secreted into the media, and ~50% of the secreted protein was biologically active (Table 1). No biological activity was detected in the cellular extracts despite the presence of factor IX polypeptide. Extracts of BHK cells had no inhibitory effect on the clotting assay.

It was necessary to supplement the cell culture medium with vitamin K at $1\text{--}10 \mu\text{g ml}^{-1}$ to detect factor IX clotting activity (data not shown). In the absence of vitamin K, the cells secreted factor IX polypeptide at somewhat higher levels than when vitamin K was present. However, without vitamin K, little or no factor IX clotting activity was detected.

Additional analyses were performed to demonstrate that these cells were secreting factor IX. Samples containing factor IX activity as measured by the above assay were incubated with factor VIII-deficient plasma; none of these samples shortened the clotting times of factor VIII-deficient plasma (data not shown). Therefore, it seemed that the activity was due to factor IX rather than an artefactual agent. This conclusion was confirmed by the finding that treatment of the samples with an affinity-purified rabbit polyclonal antibody against human

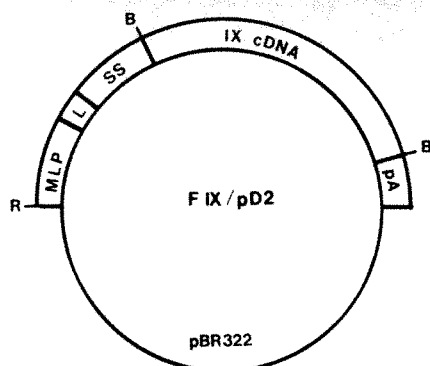


Fig. 1 Diagram of the factor IX construction used in our experiments. The factor IX cDNA obtained by Kurachi and Davie⁵ begins with the first methionine (ATG) of the protein leader sequence¹². The sequence of the 5' end was altered from CTGCAGG₁₁ ATG to CTGCAGGATCCATG to remove the G₁₁ and to introduce a *Bam*HI site 5' of the ATG. An adaptor was made by annealing two synthetic single-stranded DNAs, filling-in with T4 polymerase, and then digesting with the restriction enzymes *Pst*I and *Cfo*I. The resulting 19-bp *Pst*I/*Cfo*I fragment was ligated with a 1.4-kb *Cfo*I/*Bam*HI fragment which contained the remainder of the factor IX cDNA and a 2.7-kb *Bam*HI/*Pst*I fragment of pUC13. The DNA construction was confirmed by sequencing through the region where the adaptor was inserted. The cDNA was then isolated as a *Bam*HI fragment and inserted into the *Bam*HI site in the vector pD2 to make FIX/pD2. The pD2 plasmid (5.3 kb) was derived from plasmid pDHFR-III (ref. 13) and contains the adenovirus-2 major late promoter (MLP), the cDNA encoding the entire tripartite leader (L), a splice set (SS) composed of the adenovirus-2 third leader 5' splice site and an immunoglobulin 3' splice site, and the early SV40 polyadenylation signal (pA). R, *Eco*RI restriction enzyme site; B, *Bam*HI site.

factor IX removed 98% of the factor IX activity. This antibody also precipitated 99% of the factor IX activity from normal plasma. When the samples were immunoprecipitated with a rabbit polyclonal antibody to human erythropoietin, none of the factor IX activity was removed from the samples.

Barium citrate has been used to adsorb selectively proteins containing gla residues⁸. Culture medium from cell line IX-F was subjected to adsorption as described in Table 2 legend. The supernatant and the pellet fractions were assayed by the clotting assay and by ELISA. The ELISA data (Table 2) show that while 50% of the factor IX polypeptide was adsorbed to barium citrate, almost all of the biologically active factor IX was properly carboxylated (and therefore adsorbed to barium citrate), whereas the inactive factor IX was probably deficient in gla residues. Human factor IX isolated from plasma contains 12 gla residues⁹. Protein sequence and amino-acid composition analyses of our active and inactive factor IX fraction will be necessary to determine precisely the extent of carboxylation in the factor IX produced by BHK cells.

A Western blot analysis was performed on the material adsorbed to barium citrate from spent media of cell line IX-F and BHK cells, and from normal plasma. The protein secreted by IX-F (Fig. 2, lane 2) migrated with the same apparent M_r as the single-chain form of factor IX in normal plasma (lane 3) and of purified factor IX (lane 4). These results indicate that the cells secreted human factor IX of the correct size and that the single-chain form was stable in the cell culture medium for up to 6 days.

The present work demonstrates that biologically active human factor IX can be produced in BHK cells. Additional studies are in progress to increase the levels of protein secretion and to optimize the expression of active factor IX in transfected cells. Since variation has been observed in the rate of carboxylation by microsomal fractions from different tissues¹⁰, one parameter

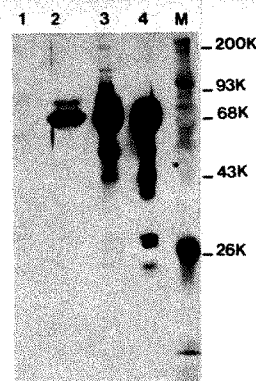


Fig. 2 Western blot of factor IX adsorbed to barium citrate. Spent media were collected from BHK cells and from IX-F cells grown for 6 days as described previously. Aliquots (2 ml) of each medium sample and normal plasma (2 ml) were adjusted to 23.8 mM sodium citrate and incubated at 4 °C for 5 min. Barium chloride was added to a final concentration of 45.5 mM, incubated at 4 °C for 30 min and centrifuged at 3,000 r.p.m. for 2 min. The pellets were washed twice with PBS and redissolved in 2 ml of PBS containing 0.15 M sodium citrate. The IX-F sample contained 400 ng ml⁻¹ factor IX by ELISA, and the control BHK samples contained none. The samples were dialysed for 4 h at 4 °C against PBS and then concentrated using Aquacide III (Calbiochem-Behring) to a volume of 300 μ l. The control BHK sample (lane 1), the IX-F sample (75 μ l; 75 ng of factor IX) (lane 2), normal plasma (20 μ l; 400 ng of factor IX) (lane 3) and PBS (50 μ l) containing 1 μ g of purified factor IX (lane 4) were subjected to electrophoresis in a 10% polyacrylamide gel (acryl:bis, 3.75:1). After the proteins had been transferred to nitrocellulose, the filter was washed with transfer buffer A (50 mM Tris-HCl pH 7.4, 5 mM EDTA, 50 mM NaCl, 0.25% (w/w) gelatin, 0.05% NP40) and incubated with rabbit polyclonal antibody to factor IX for 1 h at room temperature. After three more 15-min washes with buffer A, the filter was incubated with goat anti-rabbit IgG conjugated with horseradish peroxidase (HRP) for 1 h at room temperature, washed once with distilled H₂O, twice with buffer B (same as buffer A but also containing 0.4% N-lauryl sarcosine sodium salt) for 15 min, and finally incubated with HRP colour reagent (Bio-Rad). The factor IX in all lanes migrated with an apparent M_r of 66,000. Presumably this difference from the reported 57,000 M_r results from the electrophoretic properties of factor IX in this system. Some degradation of the purified factor IX standard (lane 4) was apparent. The M_r standards (BRL, pre-stained with Coomassie blue) (lane M) are myosin heavy chain (200,000 or 200K), phosphorylase b (92,500), BSA (68,000), ovalbumin (43,000) and α -chymotrypsin (25,700).

Table 2 Barium citrate adsorption of factor IX from cell culture medium

Sample	Factor IX (ng) (ELISA)	Factor IX (ng) (activity)
Medium from clone IX-F	5,288	3,186
Supernatant fraction after precipitation and dialysis	2,641	14
Redissolved precipitate	1,500	1,935

Spent media were collected from cell line IX-F and assayed by ELISA and by the clotting assay. A 4.8-ml aliquot of medium was adjusted to 20 mM sodium citrate and the sample was incubated for 5 min at 4 °C. Barium chloride was added to a final concentration of 40 mM and the sample was incubated at 4 °C for 30 min and then centrifuged at 3,000 r.p.m. for 2 min. The supernatant was dialysed against PBS at 4 °C, and then assayed. The pellet of insoluble barium citrate was redissolved in PBS containing 0.1% BSA and 0.15 M sodium citrate, dialysed against PBS and then assayed. According to both the activity assay and the ELISA, the per cent recovery of protein from the barium citrate pellet in this experiment was about 60%. We found that the per cent recovery varied between experiments, but the activity and the ELISA measurements were always consistent. Spent media collected from control BHK cells were subjected to the same analysis, and no factor IX activity or ELISA-positive material was detected.

to explore is the efficiency of γ -carboxylation in other cultured cell lines. From these initial studies, however, it is clear that this approach provides the basis for establishing an abundant pure source of human factor IX.

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Isolation of a new human oncogene from a diffuse B-cell lymphoma

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Utilizing DNA transfection analysis¹ with the continuous NIH 3T3 cell line as assay cell², we³ and others^{4,5} have observed that as many as 10-50% of human haematopoietic tumours contain oncogenes, the vast majority of which are members of the *ras* proto-oncogene family. In addition, Cooper and co-workers⁶⁻⁸ have reported the detection and isolation of specific oncogenes, *B-lym* and *T-lym*, which appear to be activated in human and rodent tumours of certain B and T lymphoid cells, respectively. In surveying human haematopoietic malignancies, we observed that DNA of a primary human diffuse B-cell lymphoma induced an unusual transformed focus on transfection of NIH 3T3 cells. Here, we report the molecular cloning and physical characterization of this human oncogene, whose transforming activity was shown to reside within a human DNA sequence of 45 kilobases (kb) cloned in a cosmid vector. Its properties distinguish it from previously reported retroviral or nonretroviral oncogenes.

High relative molecular mass (M_r) DNA was isolated from the affected spleen of a patient with diffuse B-cell lymphoma. When the DNA was tested in the NIH 3T3 transfection assay, transforming activity was observed at low efficiency. The transformed cells demonstrated a very distinct phenotype (Fig. 1). At least three types of morphologically distinguishable cells were visible: giant polynucleated cells, densely growing fibroblast-like cells and very refractile and round cells (Fig. 1). The unusual phenotype of the transformed focus appeared to be a genetically stable property. Clonally derived lines retained the complex morphology of the originally observed transformed focus.

A first-cycle focus was picked and the transformant grown up to mass culture. DNA of the primary transfectant was shown to contain human repetitive sequences, indicating that the transfectant had incorporated exogenous human DNA. Transmissibility of the transformed phenotype was retained in subsequent cycles of transfection with concomitant increased efficiency (Table 1). Moreover, cell lines established from first-, second- and third-cycle transfectants induced by this human transform-

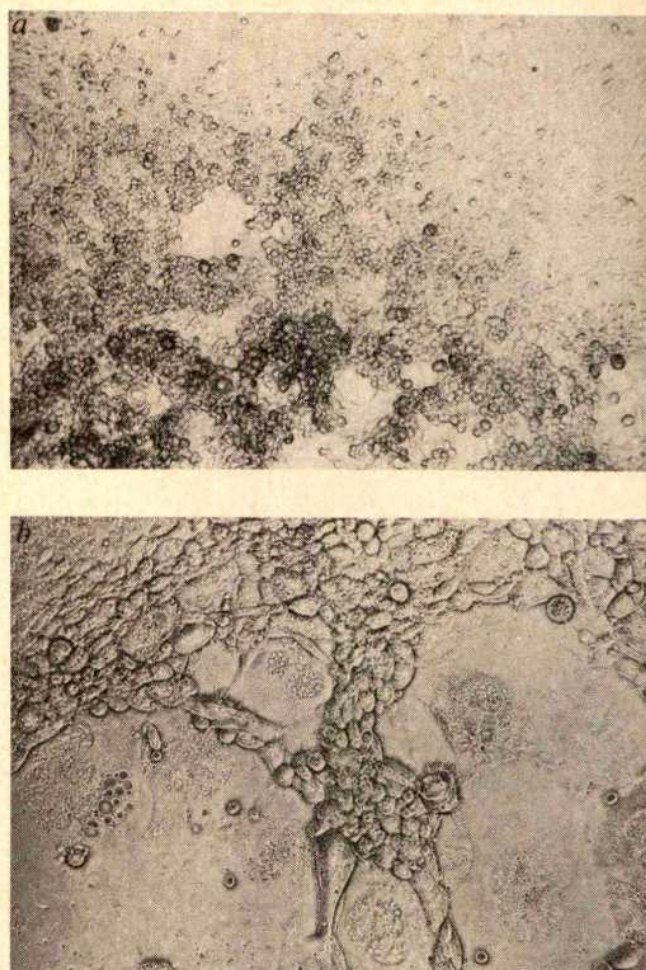


Fig. 1 Morphology of a transformed focus induced in NIH 3T3 cells by transfection of high- M_r DNA from a human diffuse B-cell lymphoma. *a*, $\times 140$; *b*, $\times 360$.

ing gene possessed known properties of malignant cells (Table 1).

We then analysed second- and third-cycle transfectants by Southern blotting analysis and hybridization with a probe specific for human repetitive sequences. As shown in Fig. 2, human sequences were detected in each transfectant analysed. The pattern of human DNA fragments observed following digestion with different enzymes indicated the retention of specific human fragments through several cycles of transfection, implying their probable association with the transforming gene.

Most human transforming genes detected by transfection analysis to date have been found to be members of the *ras* family, with *N-ras* the most frequently activated oncogene in human haematopoietic tumours³⁻⁵. To exclude the possibility that the newly detected oncogene was related to any of the known *ras* genes, we analysed transfectant DNAs by Southern blotting for the presence of human *ras*-related sequences. None of the *ras* gene probes detected related sequences other than those normally present in mouse cells (data not shown).

A third-cycle transfectant DNA was partially digested with *EcoRI*, selected by sucrose gradient for the size range of 35-45 kb and cloned into cosmid pH79. A library of 300,000 recombinants was screened with human genomic DNA as a probe. Four overlapping clones were isolated (C14-1-2, C5-1-1, C44-1-6, and C8X-1-1, Fig. 3a), whose 40-45-kb inserts contained human repetitive sequences. A preliminary restriction map was constructed and is shown in Fig. 3b. *EcoRI* and *HindIII*, which cut each of the inserts a minimum of nine times, were partially mapped, so only those *EcoRI* and *HindIII* sites that were

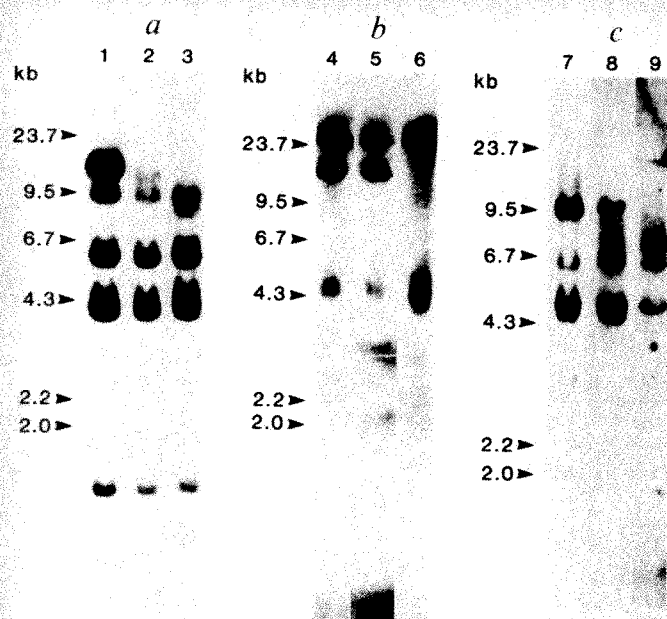


Fig. 2 Detection of human sequences in NIH 3T3 cells transformed by DNA of a diffuse B-cell lymphoma. High M_r DNA (20 μ g) was digested with *Eco*RI (a), *Bam*HI (b) or *Hind*III (c), electrophoresed, blotted and hybridized to ^{32} P-labelled nick-translated 0.3-kb cloned repetitive sequence DNA fragment purified from plasmid BLUR-8 (2×10^6 c.p.m. ml^{-1}). Cellular DNA was isolated from one representative second-cycle transfectant (lanes 1, 4, 7) and two representative third-cycle transfectants (lanes 2, 5, 8 and 3, 6, 9, respectively). *Hind*III fragments of λ DNA served as M_r standards.

determined are indicated. As shown in Fig. 3c, two of the clones, C5-1-1 and C14-1-2, contained only human sequences and were placed in the map arbitrarily at the left end of the cloned DNA sequences. The other two clones, C44-1-6 and C8X-1-1, contained both human and mouse repetitive sequences and thus probably represented one of the junctions of the human acquired sequence with mouse DNA.

To establish whether any of the clones possessed transforming activity, we transfected NIH 3T3 cells with varying amounts of each of the recombinant cosmid DNAs. C14-1-2, which contained a 45-kb human DNA fragment, was found to be biologically active in three separate experiments using two different DNA preparations with an efficiency of focus formation of about 600 focus forming units per pmol (data not shown). The morphology of transformed foci induced by the cloned oncogene was indistinguishable from that observed by transfecting NIH 3T3 with the original human tumour or transfectant DNAs.

DNAs of representative foci obtained by transfection with C14-1-2 DNA were analysed by Southern blotting using as probes a 4.5-kb *Bam*HI fragment that maps at the right end of C14-1-2 (Fig. 3a). We found that this probe recognized DNA fragments of the sizes expected from the physical map of C14-1-2 in all the transfectant DNAs analysed. All of these findings established that the C14-1-2 recombinant cosmid clone contained the biologically active transforming gene.

We hybridized third-cycle transfectant DNAs with cloned viral and cellular oncogenes and performed reciprocal hybridization studies between these probes and the cloned oncogene. In addition to its lack of homology with any of the known *ras* genes, the transforming gene lacked detectable relationship to *sis*, *rel*, *fos*, *erb-B*, *B-lym*, *N-myc*, *fgr*, *fes*, *src*, *ets*, *fms*, *p53*, *myb*, *mos*, *abl*, *myc* and *raf* (data not shown). Furthermore, its restriction map was different from that of *T-lym*⁸ as well as that recently reported for *met*⁹. Finally, comparison of the pattern of *Alu*-containing fragments of second- and third-cycle transfectants with the human specific fragments of *met*⁹, *mcf-2*¹⁰, *mcf-3*¹⁰,

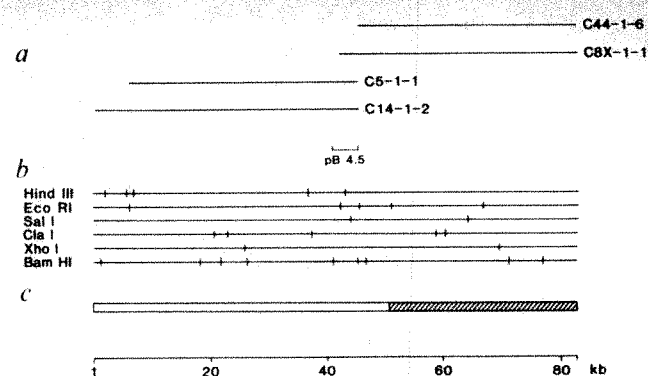


Fig. 3 Restriction map of *dbl*. a, Genomic DNA of a third-cycle transfectant was partially digested with *Eco*RI to select for fragments of from 35–45 kb and cloned in pHC79 cosmid cloning vector. The library was screened without amplification with ^{32} P-labelled nick-translated, sheared human genomic DNA as a probe. The four overlapping recombinant clones obtained are indicated. A *Bam*HI restriction fragment of cosmid clone C14-1-2 was subcloned in pAT153 (subclone pB 4.5). b, Restriction map of the cloned cellular DNA. *Hind*III and *Eco*RI were only partially mapped. c, Human (open box) and mouse (hatched box) specific sequences were determined by hybridization with ^{32} P-labelled nick-translated, human genomic DNA, human *Alu* clone BLUR-8 and mouse genomic DNA.

NK14¹¹ and *neu* oncogenes¹² indicated a lack of homology between our transforming gene and any of these recently detected oncogenes.

Our present studies provide the first report of a transforming gene associated with a human diffuse B-cell lymphoma. This newly identified oncogene, which we designate *dbl* for diffuse B-cell lymphoma, conferred the transformed phenotype on NIH 3T3 cells, inducing an unusual focus composed of small and spindle-shaped refractile cells as well as large multinucleated giant cells. Despite the low transforming activity of the original human tumour DNA, we presume that the transforming gene arose from within the tumour, as we have not observed transformed foci induced by normal human DNA in a large series of transfection experiments. However, direct confirmation must await further characterization of the structure of the oncogene as it resides within the tumour. The distinct phenotype

Table 1 Transforming properties of DNA from a human diffuse B-cell lymphoma

Transforming activity		Properties of transformed cells		
Donor DNA	No. foci per plate*	Saturation density (cells $\times 10^{-5}$ per cm^2)†	% Colony formation in agar‡	Tumour incidence§
I cycle	0.12	2.0-3.0	5-10	5/5
II cycle	1.5	2.0-3.0	5-10	5/5
III cycle	3.0	2.0-3.0	5-10	5/5
Control NIH 3T3	<0.03	1.0	<0.01	0/5

* 1.5×10^5 NIH 3T3 were transfected with 40 μ g of high M_r DNA by the calcium phosphate method¹. Results represent mean values of at least eight recipient cultures.

† The maximum cell number obtained when 5×10^3 cells per cm^2 were inoculated onto 20-cm² Petri dishes in conditions where the medium containing 5% calf serum was changed every 3 days. Saturation density was taken as that value where successive cell counts at 2-day intervals showed no increase in cell number.

‡ Cell suspensions were plated at 10-fold serial dilutions in 0.33% soft agar medium containing 10% calf serum. Visible colonies comprising >100 cells were scored at 14 days.

§ NFR nude mice were inoculated subcutaneously with 1×10^5 cells of each cell line. Tumour formation was monitored for up to 30 days.

of transformants induced by this oncogene suggests that the NIH 3T3 transfection assay may reveal other oncogenes which induce alterations that differ from those caused by *ras* oncogenes.

Our isolation of a 45-kb cosmid clone comprised of human sequences, which induced transformation on DNA transfection, demonstrates that the transforming gene was obtained in a biologically intact form. This clone was only one of four isolated by cosmid cloning of *Eco*RI partially digested genomic DNA. Thus, it is likely that restriction sites outside the transforming region were preferentially cleaved by the cloning enzyme. To our knowledge this 45-kb DNA sequence represents the largest biologically active transforming DNA so far isolated.

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Control of transcription of α -amylase and rRNA genes in barley aleurone protoplasts by gibberellin and abscisic acid

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Gibberellin A₃ (GA₃) promotes and abscisic acid (ABA) inhibits the production of α -amylase in aleurone layers of barley (*Hordeum vulgare* L.); this system has been used extensively to study the mechanisms of action of these hormones¹. Cell-free messenger RNA translation studies have shown that the GA₃-promoted increase in α -amylase synthesis and its inhibition by ABA are associated with corresponding changes in the levels of translatable α -amylase mRNA²⁻⁵. Subsequent studies using hybridization of an α -amylase complementary DNA-rich probe⁶ and of cloned α -amylase cDNA probes to aleurone RNA⁷⁻⁹ have shown that the abundance of α -amylase mRNA increases in response to GA₃, and that this increase is inhibited by ABA. The increased abundance of mRNA could result from an increased rate of transcription, increased stability of mRNA, or both, as in some animal cells¹⁰⁻¹². By using methods for studying gene transcription in isolated plant nuclei¹³⁻¹⁵, we present here evidence that GA₃ and ABA regulate the transcription of both α -amylase and ribosomal RNA (rRNA) genes

Nuclei were isolated from GA₃-responsive aleurone cell protoplasts¹⁶ and to facilitate this, several minor changes were made to our earlier protoplast preparation procedure. Figure 1 shows that, using the modified procedure, very little α -amylase activity accumulated in control protoplasts, that there was a marked

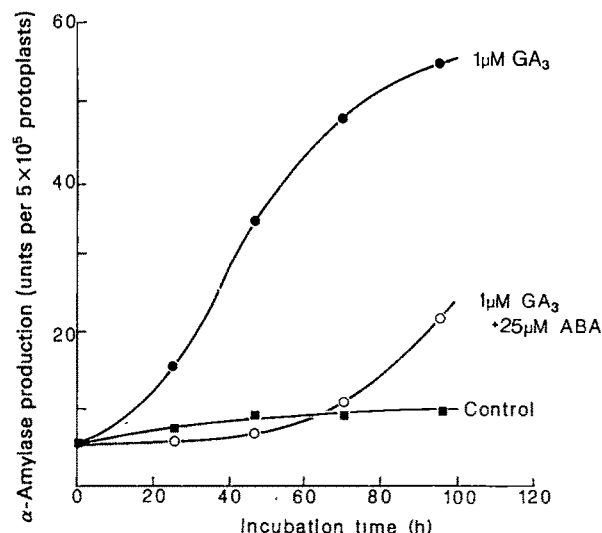


Fig. 1 Regulation of α -amylase produced by barley aleurone protoplasts in response to GA₃ and ABA

Methods. Protoplasts were prepared from aleurone layers of barley (*H. vulgare* cv. Himalaya) under nitrogen as in an earlier study¹⁶ but 20 quarter grains, not 10, were placed in each Erlenmeyer flask and the volume of protoplast isolation medium containing cellulase Onozuka R-10 was increased from 1 ml to 1.5 ml, this procedure gave about 500,000 protoplasts per flask. Flasks received 100 μ l of either water (control, ■) or a solution of GA₃ (final concentration 1 μ M, ●) or GA₃ plus *cis*, *trans*-ABA (final concentration 25 μ M) (○) and were left without shaking at room temperature ($22 \pm 2^\circ\text{C}$). Assay and expression of α -amylase activity have been described elsewhere¹⁶. Values represent the mean of four flasks of protoplasts.

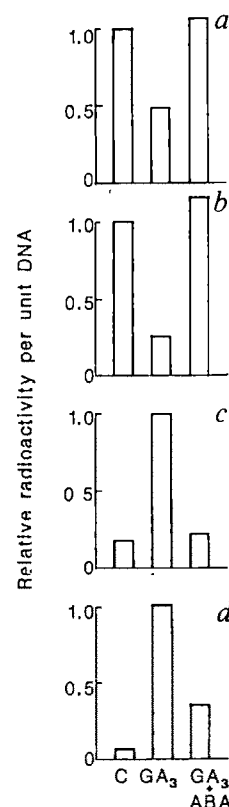


Fig. 2 Comparison of the effects of hormones on accumulation of total (a) and various specific transcripts (b-d) in isolated nuclei. b, rRNA; c, α -amylase; d, RNA specified by pHV14. The relative values for each RNA species were calculated from the values of c.p.m. per 100 μ g DNA given in Table 1. c, control nuclei; GA₃, nuclei from GA₃-treated protoplasts; GA₃ + ABA, nuclei from protoplasts treated with both GA₃ and ABA.

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Table 1 Accumulation of transcripts by nuclei isolated from barley aleurone protoplasts incubated for 24 h with and without GA₃ and ABA

	Control	GA ₃	GA ₃ + ABA
Total transcription	7,530 (100)	3,712 (100)	8,075 (100)
rRNA	1,355 (18)	360 (9.7)	1,574 (19.5)
α -Amylase transcripts	0.25 (0.0033)	1.37 (0.037)	0.32 (0.0040)
pHV14 transcripts	0.17 (0.0023)	2.52 (0.068)	0.89 (0.011)

The values are (c.p.m. per 100 μ g DNA) $\times 10^{-3}$ and the numbers in parentheses are percentages of total transcription. Protoplasts were prepared and incubated for 24 h with and without hormones as described for Fig. 1 (10 flasks per treatment). Because starch grains interfered with the protoplast lysis procedure (see below), protoplasts were purified on discontinuous Percoll gradients made essentially as described by Luthe and Quatrano¹⁹. The gradients consisted of 5-ml layers of 2 M sucrose (bottom), and of 80% and 15% Percoll in 30-ml Corex centrifuge tubes. The Percoll was made up in 0.44 M sucrose (RNase-free, Bio-Rad), 25 mM Tris-HCl pH 7.5 and 10 mM MgCl₂. The contents of five incubation flasks were layered onto each gradient (on ice) and the tubes were centrifuged at 4,080g (5,000 r.p.m. in a Sorvall HB4 swinging bucket rotor) for 20 min. The protoplasts band at the top and bottom of the 15% Percoll layer, the starch falls to the bottom of the tube, and the dead protoplasts float to the top of the original protoplast medium. The entire 15% Percoll layer was recovered and diluted with an equal volume of 0.44 M sucrose, 25 mM Tris-HCl pH 7.5, 10 mM MgCl₂, 5% w/v Ficoll and 10% w/v Dextran 40. To this mixture was added 1 μ l ml⁻¹ β -mercaptoethanol, 0.5% (v/v) Triton X-100 and 2 mM spermine; the cells were then contained in a solution similar to the modified Honda medium of Luthe and Quatrano¹⁹. The protoplasts were broken by passing the suspension five times through nylon mesh with nominally 20- μ m pores (actually 13 μ m when measured). The nuclei were recovered from solution by centrifugation (3,000 r.p.m. for 10 min in an HB4 rotor) and washed twice in 10 ml of 2.5% Ficoll 400, 5% Dextran T40, 0.25 M sucrose, 0.025 M Tris-HCl pH 7.8, 0.01 M MgCl₂ and 1 μ l ml⁻¹ β -mercaptoethanol. Pellets were resuspended gently using a glass rod and centrifuged at 3,000 r.p.m. in an HB4 rotor for 5 min. The washed pellet derived from 10 flasks of protoplasts was resuspended in ~150 μ l (total volume) of the washing buffer. Transcription reactions were started by adding 120 μ l of nuclei (usually containing ~80 μ g DNA) to 600 μ l of transcription mixture. Final concentrations of components were: 50 mM (NH₄)₂SO₄, 0.45 mM each of ATP, GTP and UTP, and 2 mM MnCl₂. Each reaction contained 300 μ Ci [α -³²P]UTP (specific activity ~3,000 Ci mmol⁻¹; Amersham). Attempts to use [α -³²P]GTP to radiolabel RNA were unsuccessful because transcription was progressively more inhibited as the amount of radioactive GTP in the reaction was increased. The reactions were incubated at 20 °C for 1 h. To measure accumulation of total RNA, aliquots of the reaction mixture were taken at the beginning and end of the reaction and the nucleic acid was precipitated with cold trichloroacetic acid (TCA) in the presence of carrier herring sperm DNA and the radioactivity in RNA determined as described previously¹⁷. To measure specific gene transcripts, the RNA was first extracted from the nuclei. The nuclei, containing 94% of the TCA-precipitable radioactivity, were recovered from the reaction mixture by centrifugation and the RNA was purified essentially as described elsewhere¹⁷, except that the proteinase K and sonication steps were omitted. The ethanol-precipitated nucleic acid was centrifuged and dissolved in 50 μ l of 0.1% SDS, 0.5 mM EDTA and 1 mM Tris-HCl pH 7.5. Recombinant plasmids used to detect specific transcripts were pTA71, containing wheat genomic ribosomal DNA²⁰; pHV19, containing sequences for barley aleurone α -amylase⁹, and pHV14, which is unidentified but contains sequences for a barley polyadenylated RNA species whose concentration increases in the presence of GA₃, as does α -amylase mRNA⁹. The control plasmid was pP15-71, which contains *Psidium sativum* cDNA sequences specific for vicilin²¹. Plasmid DNA (2 μ g) was bound to nitrocellulose filter disks (6.5 mm diameter) by the method of Thomas²². Hybridizations were performed as described previously¹⁷, except that rRNA hybridizations were done in 30 μ l and the others in 100 μ l. All hybridizations contained a pP15-71 filter for the determination of background radioactivity, which was subtracted from that binding to other filters in the hybridization. Hormone treatments did not affect the recovery of DNA from protoplasts but, because they did affect total transcription, hybridized counts have been expressed both as a percentage of total transcriptional activity and as c.p.m. per 100 μ g DNA ($\times 10^{-3}$), as described previously¹⁷.

response to 1 μ M GA₃ and that a 25-fold excess of ABA over GA₃ strongly inhibited α -amylase accumulation, at least up to 48 h, at which time accumulation of the enzyme appeared to be released from ABA control. From these results, we chose to isolate nuclei for *in vitro* transcription experiments from protoplasts incubated with or without hormones for 24 h, as at this time, ABA is strongly inhibitory and the rate of α -amylase accumulation is still increasing. Previous studies indicated that an increasing rate of α -amylase accumulation is associated with increasing levels of α -amylase mRNA^{2,5}. If the increasing levels of α -amylase mRNA are the result of increased transcription of the α -amylase genes, then 24 h should be a suitable time to detect it.

Initially, nuclei were isolated from protoplasts which had been incubated for 24 h with and without GA₃ and ABA (as in Fig. 1) and their capacity for transcription *in vitro* was determined. All nuclei preparations incorporated nucleoside triphosphates into RNA for 1 h, after which acid-precipitable RNA was slowly lost, presumably as a result of ribonuclease activity (data not shown). Accordingly, all transcriptions were run for 1 h, at which time total incorporation of [α -³²P]UTP into RNA reached maximal levels of up to 80,000 c.p.m. per μ g DNA, depending on the treatment (see below).

To determine the effects of hormones on the accumulation of total transcripts and on the accumulation of specific gene transcripts in isolated nuclei, protoplasts were incubated with or without hormones for 24 h, and their nuclei were isolated and allowed to transcribe *in vitro*. The *in vitro*-synthesized RNA was hybridized to specific cloned DNAs (see Table 1). The results in Table 1 are from one experiment but they are representative

of results obtained in two other experiments. Incorporation of [α -³²P]UTP into total RNA was decreased in nuclei isolated from protoplasts treated with GA₃ compared with the level in nuclei from control protoplasts. This decreased incorporation did not occur when protoplasts were treated with ABA in addition to GA₃ (Table 1). Because the concentration of UTP added to the transcription reaction was very low (0.12 μ M), these results could be explained by hormone-mediated effects on the size of the residual UTP pool in isolated nuclei. However, experiments using UTP concentrations 77 times greater showed the same effects of the hormones on total RNA accumulation. Thus, the hormonal effects are not artefacts resulting from changes in specific radioactivity of the *in vitro*-synthesized RNA.

In the above experiment, the rRNA transcripts contributed 18% of the total transcripts in nuclei from untreated protoplasts although in other experiments their contribution was as low as ~6%. The range of these values is similar to that reported for pea leaf nuclei¹³ but considerably less than the range for pea cotyledon nuclei, which was ~45 to 77% (ref. 17). The accumulation of rRNA transcripts in this and other experiments was depressed by about 50% in protoplasts treated with GA₃ but this did not occur if ABA was also present.

The hormones also had large effects on the accumulation of α -amylase gene transcripts. In accordance with the very low level of α -amylase activity and α -amylase mRNA in control protoplasts¹⁶, there was very little accumulation of α -amylase gene transcripts in control nuclei; when GA₃ was present, however, accumulation of the transcripts increased about 11-fold to a level of 0.037% of total transcription. Incubation of proto-

plasts with ABA in addition to GA₃ once again inhibited the effect of GA₃. The α -amylase cDNA clone pHV19 probably corresponds to mRNA for the B group of α -amylase isozymes⁹. We believe that in the hybridization conditions of the present experiments, transcripts of both A and B α -amylase families will hybridize to pHV19 because of the relatively high nucleotide sequence homology between group A and B cDNA clones and because of their high G+C content⁹. Therefore, the α -amylase gene transcripts measured here probably represent the composite contribution of several α -amylase genes, estimated to be at least eight in the *H. vulgare* cv. Himalaya genome^{7,9}. Our results do not allow us to determine whether or not GA₃ regulates the transcripts of all genes equally.

We also measured the accumulation of transcripts specific to clone pHV14 because it specifies a mRNA whose abundance also increases in the presence of GA₃, as does α -amylase mRNA, although we have not identified any protein that it encodes⁹. The effects of GA₃ and ABA on the synthesis of RNA hybridizing to pHV14 were similar to their effects on α -amylase gene transcription (Table 1).

These results leave little doubt that GA₃ and ABA regulate nuclear activity in barley aleurone cells. The most likely explanation for the results is that the hormones regulate specific gene transcription and thus mRNA formation, although it is still possible that altered transcript stability and processing within the nucleus may be involved. The accumulation of α -amylase gene transcripts was increased about six times in nuclei from GA₃-treated protoplasts although in other experiments it was increased by up to 14 times. The variation in the extent of the increase of α -amylase gene transcripts with GA₃ treatment probably reflects the small and variable numbers of hybridizable counts in RNA accumulated by control nuclei. It is of interest to examine whether the hormone-promoted increase of α -amylase transcripts could account for the observed changes in level of α -amylase caused by GA₃. The rate of α -amylase accumulation after 24 h was increased from ~0.053 units h⁻¹ to ~0.63 units h⁻¹, an increase of a factor of ~12 (Fig. 1). Although these values are approximate, the increased concentration of α -amylase gene transcripts is clearly in accord with the increased rate of α -amylase accumulation. However, more detailed studies of rates of gene transcription, mRNA levels, mRNA stability and rates of enzyme synthesis are necessary to determine whether GA₃ controls α -amylase synthesis solely by regulating gene transcription.

A review of the literature shows that ABA has been found to reverse all of the GA₃-promoted changes in protein synthesis or mRNA levels in barley aleurone, whether they be increases or decreases¹. The results of the present study add to that list. ABA not only prevents the GA₃-promoted accumulation of α -amylase gene transcripts and of pHV14 transcripts in nuclei, but also prevents the suppression of total transcripts and of ribosomal transcripts by GA₃. Thus, the action of ABA leads to both promotion and inhibition of transcription and in this respect resembles GA₃. In summary, these experiments indicate that the inhibitory effect of ABA on α -amylase synthesis and on the accumulation of α -amylase mRNA²⁻⁹ can be accounted for largely by its effects within the nucleus, probably on gene transcription. A cytoplasmic role for ABA, in particular at the level of translation¹, in the conditions of these experiments would appear to be unnecessary.

These results also exemplify the complexity of GA₃ action. GA₃ has multiple effects in cereal aleurone, increasing the concentration of some mRNAs and proteins and decreasing the concentration of others^{5,18}. Here against a background of reduced total transcripts, GA₃ caused suppression of ribosomal gene expression and an increased expression of two other genes (Fig. 2). It seems, therefore, that the multiplicity of effects elicited by GA₃ on protein and RNA levels is also seen at the level of transcription.

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Cooperative dimeric and tetrameric clam haemoglobins are novel assemblages of myoglobin folds

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Cooperative functioning of many protein systems depends on communication between different subunits of those systems. Perhaps the best understood cooperative protein system is the vertebrate haemoglobin tetramer, in which the subunits share a similar tertiary structure (the myoglobin fold) with each other and with myoglobins and haemoglobins from at least four different animal phyla and leguminous plants. Blood clams have cooperative tetrameric haemoglobin and, in addition, a cooperative homodimeric haemoglobin¹⁻⁶. In view of previous reports^{7,8} concerning the role of dimers in the vertebrate tetramer, the clam haemoglobins represent a very interesting model system. We report here the low-resolution three-dimensional crystal structures of the dimeric and tetrameric cooperative haemoglobins from the blood clam *Scapharca inaequivalvis*. We find that clam haemoglobins are made of myoglobin-like subunits but their assembly to form dimers and tetramers is quite different from that of vertebrate haemoglobin. The arrangement of the subunits provides a simple structural explanation for haem-haem interaction in the dimer and tetramer.

The haemoglobins in *S. inaequivalvis* are formed from three distinct polypeptide chains, two of which associate to form heterotetramers (A₂B₂), while a third associates to form homodimers⁵. The dimers and tetramers bind oxygen cooperatively with Hill coefficients of 1.5 and 2.1, respectively⁶. The dimer shows no oxygen-linked subunit association phenomena, whereas the tetramer can associate to form higher polymers in the deoxy state⁵. Although complete amino-acid sequences are not available for the haemoglobins from *S. inaequivalvis*, the sequences of two haemoglobin chains from related clams have been determined^{9,10}; these sequences align functionally with sequences from other haemoglobins that possess the myoglobin

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Fig. 1 Composite sections from the 5.5-Å clam tetramer map (A, B, D, E), with equivalent sections from a 5.5-Å map of the α -chain of human CO haemoglobin (C, F). The Fourier coefficients for the map of the human α -chain were calculated from atomic coordinates obtained from the Protein Data Bank¹⁸ with an overall B value of 20 Å². A, B, the haem and E and F helices of the two distinct subunits in the clam tetramer map; C, the equivalent region from the structure factor map of the human α -chain. These density 'slabs' cover four sections or ~ 5.5 Å perpendicular to the plane of the paper. D, E, the G and H helices of the two distinct subunits in the clam tetramer map, F, those of the human α -chain. These density slabs cover six sections or ~ 9.2 Å perpendicular to the plane of the paper. Note particularly the additional electron-dense rod in the upper portion of D and E corresponding to the pre-A helix which is present in clam haemoglobin but not in other haemoglobins. The G helix density shown in E is the poorest density obtained for a helix in the tetramer map (The G helix is on the outside of the molecule in the clam tetrameric haemoglobin).

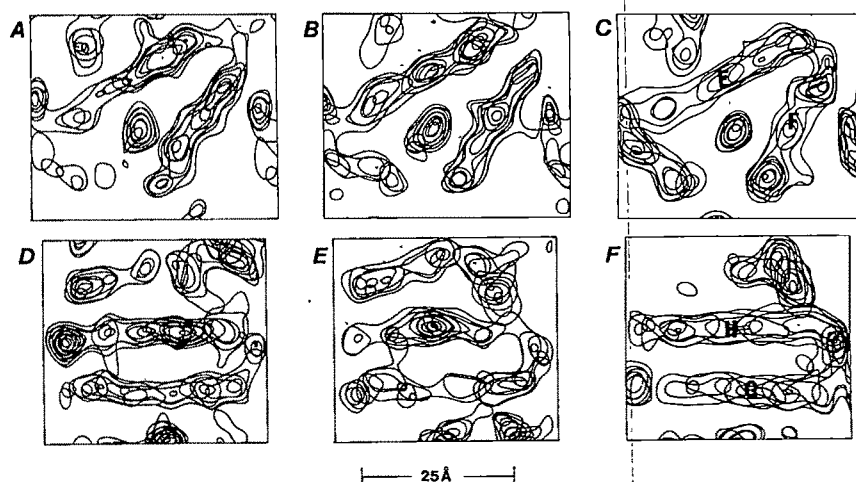
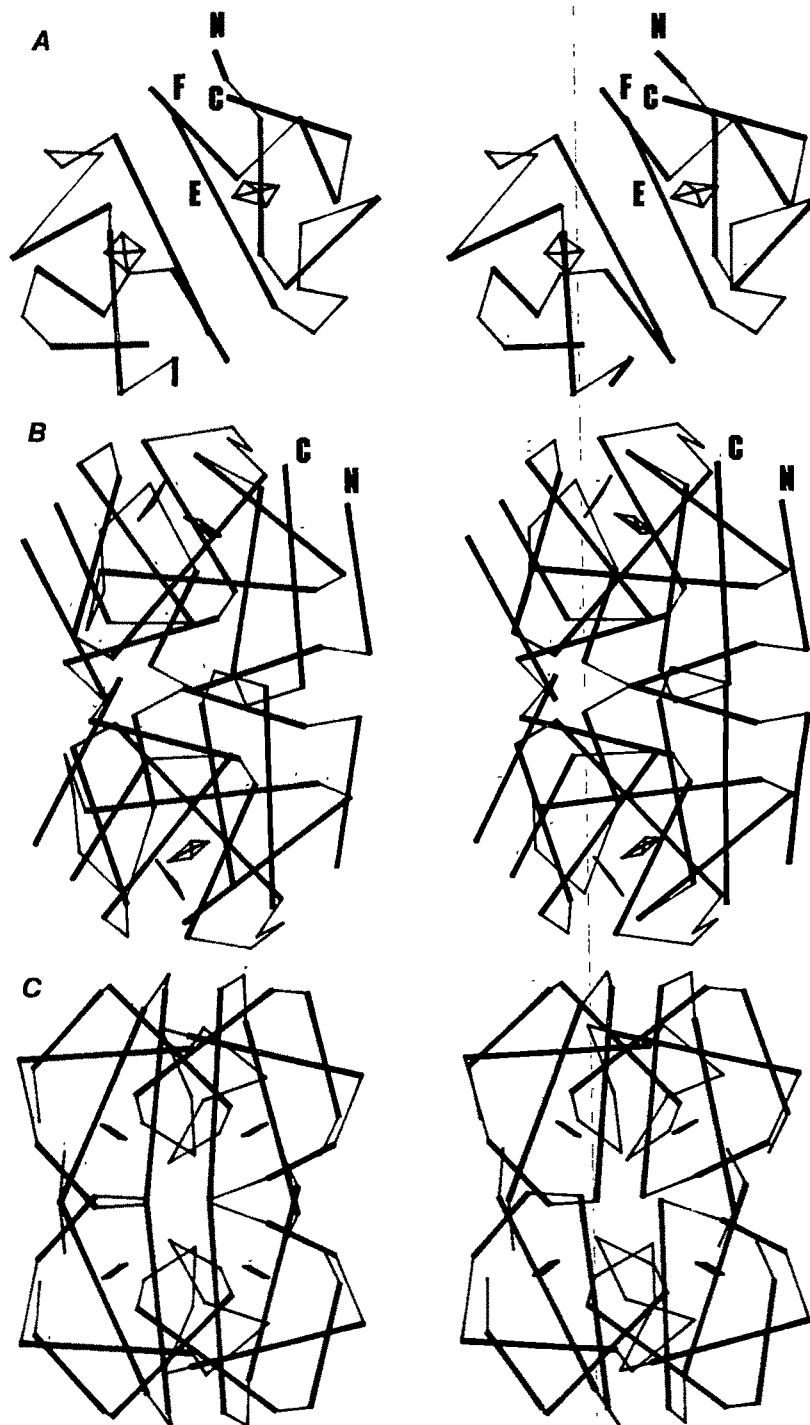


Fig. 2 Stereo diagrams showing arrangement of subunits in the clam haemoglobin dimer (A), clam haemoglobin tetramer (B) and human haemoglobin tetramer (C). The pre-A, A, B, E, F, G and H helices are drawn with heavy lines. The diagrams for the clam haemoglobins were drawn using helix end points taken from the tetramer map. The human CO haemoglobin diagram uses the α -carbon positions of residues at the end of helices, obtained from the Protein Data Bank coordinates. One subunit in both the clam dimer and tetramer is labelled N at the amino terminus and C at the carboxy terminus. A, clam dimer with the dyad relating the two subunits oriented perpendicular to the plane of the paper. Note how the E and F helices of the two subunits lie across each other to form an extensive contact. (The E and F helices are labelled in one subunit.) The slightly different chain path for the two subunits in the homodimer diagram is the result of the molecular replacement model and does not represent any real difference between the subunits. B, clam tetramer with the molecular dyad (used in symmetry averaging) oriented horizontally in the plane of the paper and the pseudo-dyad relating the two subunits within a dimer, also approximately in the plane of the paper. C, human CO haemoglobin tetramer with the molecular dyad lying horizontally in the plane of the paper and the pseudo-dyad relating α_1 to β_1 lying vertically in the plane of the paper. In vertebrate haemoglobins the pseudo-dyad is perpendicular to the molecular dyad and together they generate a third dyad. Thus, vertebrate haemoglobin has approximate 222 point group symmetry. In the clam tetramer these dyads are not perpendicular, a third perpendicular dyad is not generated, and thus the clam tetramer does not have approximate 222 point group symmetry.



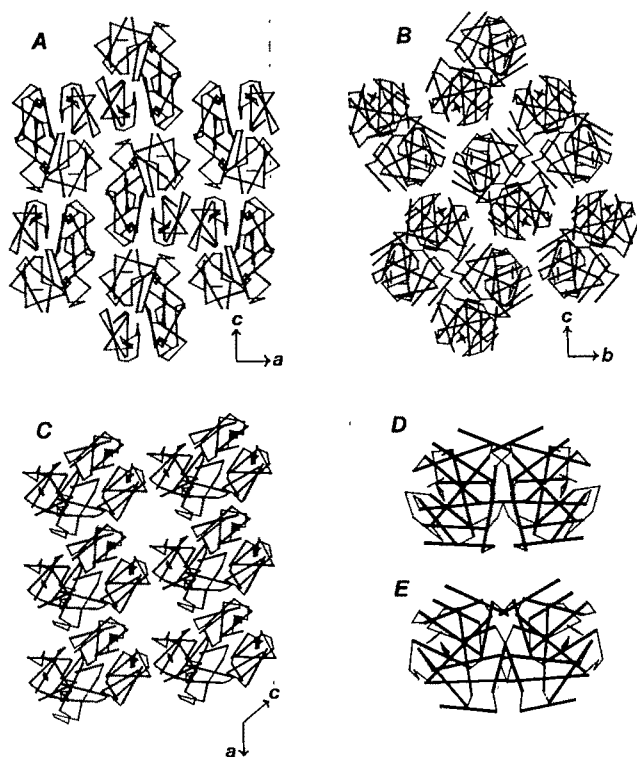


Fig. 3 The packing of dimeric and tetrameric clam haemoglobins in their crystals. Line drawings of the subunits as in Fig. 2. *A*, seven tetramers projected down *b*; the most extensive contact is between the central tetramer and the one at the lower right. *B*, seven tetramers projected down *a*. A fairly extensive contact can be seen between the central tetramer and that at the upper right, which is related to it by a crystallographic dyad axis. The space group symmetry and packing considerations are incompatible with these contacts being intramolecular. *C*, twelve dimers projected down an axis 30° away from *b*. The *b* axis is oriented into the paper, to the right. The tight association occurs across the crystallographic dyad axis along *b*. *D*, close-up of two dimers related by the crystallographic dyad along *b*, which is vertical in the figure. *E*, close-up of the tetramer with the molecular dyad vertical. Note that the dimers associate in dimer crystals much as do the half-molecules in the tetramer, but the two dimers are slightly less tightly packed than are half-molecules in the tetramer. The distances between iron atoms in neighbouring dimers are 47.5–52.5 Å, whereas the corresponding distances in the tetramer are 45.5–49.1 Å.

fold, and, in common with the hagfish, lamprey and shark, they have additional residues at the amino terminus.

The haemoglobin used in the present study was provided by Dr E. Chiancone (University of Rome). Table 1 shows data on crystal preparation and lattice constants for both dimeric and tetrameric carbon monoxide haemoglobins. Both types of crystal are robust in the X-ray beam and diffract well past 3 Å resolution.

A 5.5-Å electron density map of the tetramer crystals was calculated using phases obtained from isomorphous and anomalous differences for uranyl acetate and gold cyanide derivatives and from isomorphous differences for a potassium *cis*-dichlorodiamine platinate derivative. The 'phasing power' ($\langle f_H \rangle / (\text{error of closure})$) for the uranium, gold and platinum derivatives was 2.2, 1.6 and 1.1, respectively. The mean figure of merit was 0.79. This map contained many electron-dense rods, presumably corresponding to α -helices. The probable molecular envelope was established by considering the relatively empty solvent regions in combination with the packing constraints imposed by the location of the symmetry elements of the space group. Tentative iron positions, which subsequently proved to be correct, were identified by high electron density and small but consistent peaks in anomalous difference Fourier

Table 1 Dimeric and tetrameric crystal growth and lattice data*

	Buffer	pH	Space group	<i>a</i>	<i>b</i>	<i>c</i>	β	<i>z</i>
Dimer	2.5 M PO ₄	7.5	C2	93.3	44.1	83.6	122.0	1
Tetramer	2.4 M PO ₄	5.8	C222 ₁	102.1	94.1	128.0	—	1

* *z*, The number of molecules per asymmetric unit, *a*, *b* and *c*, Å, β , degrees

maps for both the gold and uranium derivatives. Perhaps most importantly, we found a non-crystallographic molecular dyad relating the two gold sites, two minor uranium sites and most of the electron density in its neighbourhood. Eleven cycles of symmetry averaging about this dyad dramatically improved the quality of the map. The *R*-value for the final symmetry-averaged map was 21.4% ($R = \sum |F_o - F_c| / \sum F_o$). The details of the determination of the tetramer and dimer structures will be published elsewhere.

In the final symmetry-averaged map, four myoglobin-fold subunits are evident. Each polypeptide chain can be followed with, at most, three short breaks. Each subunit contains an ellipsoid of very high density between the E and F helices, which corresponds to the haem group (see Fig. 1). An additional electron-dense rod ~ 18 Å long extends from the end of the A helix to the amino terminus of each chain; we believe this to be a helix and call it 'pre-A'. The pre-A helix is roughly parallel to, and in the same plane as, the G and H helices (see Fig. 1). It runs between the E–F corner and the H helix, and, in all subunits, the amino terminus is within 10 Å of the carboxy terminus. In retrospect, the pre-A helix made interpretation of the initial electron density maps difficult because in no other myoglobin folds do subunits have three roughly parallel helices. Other than the pre-A helix, the subunits have standard myoglobin folds.

The most striking feature of the clam tetramer structure is the manner in which the subunits are assembled. In vertebrate tetrameric haemoglobins, the G and H helices are on the inside of the molecule and are involved in subunit interactions, whereas the E and F helices are on the outside of the molecule and are exposed to solvent. However, in the clam haemoglobin tetramer, all the G and H helices are on the outside of the molecule and are exposed to solvent, whereas the E and F helices are involved in subunit interactions. Thus, the clam tetramer is 'back-to-front' relative to the vertebrate haemoglobins (see Fig. 2). The tetramer is a dimer of dimers with one dimer related to the other by the molecular dyad mentioned above. Within a dimer, the E–haem–F surfaces of the subunits face each other, forming an extensive contact.

The dimer contact involving the F helix invites speculation about the molecular basis of the cooperativity. In the deoxy form a contact similar to that seen in the liganded structure may clamp the subunits in a low-affinity state. In human haemoglobin, the F helix is displaced ~ 1 Å on CO binding¹¹, while in sperm whale myoglobin, the F helix is displaced by ~ 0.3 Å on oxygen binding¹². If displacements of the F helix occur in the clam haemoglobin subunits, binding of oxygen to one subunit would disrupt the deoxy contacts and allow the molecule to 'relax' to a partially liganded structure in which the unliganded haems have a higher affinity than those clamped in the deoxy structure.

Before the determination of the tetramer structure, we had little success with the dimer crystals. Lack of sufficient dimer crystals limited our heavy-atom search and we did not obtain any satisfactory molecular replacement solutions using various search molecules. However, on observing the back-to-front mode of association of the dimers in the tetramer structure, we speculated that the cooperative homodimer might be assembled similarly. A dimer constructed in this way could be cooperative, and from a balsa-wood model it appeared that the contacts were sufficiently extensive to be stable. In addition, a comparison of the sequences of a tetramer chain from *Anadara trapezia*⁹ with those of the dimer chain from *Anadara broughtonii*¹⁰ shows that although only 42% of the residues are identical in both chains,

84% of the residues in the E and F helices are identical. This result implies that the residues in the E and F helices are conserved between the tetramer and dimer chains for some important purpose such as contact formation.

Thus, we decided to use the dimer electron density from our tetramer map as a search molecule in molecular replacement. Structure factors obtained from the inverse Fourier transform of the isolated density were used in rotation¹³ and translation¹⁴ function calculations against the dimer data. Unlike our previous molecular replacement calculations, these gave good solutions.

We tested the validity of this molecular replacement solution using various criteria. First, the *R*-value of 42.8% compares favourably with other successful solutions^{15,16}. Second, dimer placement in the crystal showed no interpenetration between neighbouring molecules even though packing considerations were not included in the translation function (see Fig. 3). In addition, standard difference and 'haem-less'¹⁵ difference Fourier maps were calculated, examined and found to support this solution. Finally, an anomalous difference Patterson¹⁷ for native dimer crystals contained peaks at all positions for the expected iron-iron vectors.

The 'cooperative dimer' hypothesis of Antonini⁷ and colleagues in the late 1960s has been shown to be inapplicable to vertebrate haemoglobin⁸, in which the uncooperative $\alpha_1\beta_1$ dimer appears to move as an essentially rigid body. However, dimeric clam haemoglobin is cooperative, and a probable structural basis for that cooperativity has been revealed in the crystal structure. Because the same mode of subunit assembly occurs in the tetramer, it is likely that Antonini's cooperative dimer is

the underlying cause of cooperativity also in tetrameric clam haemoglobin.

To ascertain how these structures change with the release of ligands and why the dimers do not form tetramers in solution, we intend to determine the structures of liganded and deoxy dimeric and tetrameric clam haemoglobins at high resolution.

We thank Drs Emilia Chiancone and Eraldo Antonini for drawing our attention to these interesting proteins and for providing us with purified dimeric and tetrameric haemoglobins; and Drs Janet Smith and Wayne Hendrickson for the symmetry-averaging programs and many helpful discussions. This work was supported by NIH grant AM 02528. W.E.R. was supported in part by NIH training grant GM 07231.

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MATTERS ARISING

A complex of copper (II)-montmorillonite with a modified cyclodextrin

IN the article by Kijima *et al.*¹, structural organization of intercalated mono-(6- β -aminoethylamino-6-deoxy)- β -cyclodextrin (CDen) has been deduced from the interpretation of electron paramagnetic resonance (EPR) spectra of oriented film samples (CDen-Cu(II)-montmorillonite).

However, the invariance of the EPR spectrum towards the variation of the angle between the film plane and magnetic field is indicative of a random orientation of the adsorbed Cu(II) complexes rather than a spatial organization. For both models proposed in Fig. 3a and b, there should be a pronounced angular dependence of the spectrum with a maximum change at 45° in 'structure a' and a vanishing component at 90° in 'structure b'.

Since the statements in this article are inferred from an erroneous spectrum interpretation, the conclusions about the lipid bilayer orientation remain questionable.

HERBERT MOTSCHI

Eidgenössische Technische
Hochschulen,
EAWAG, CH-8600 Dübendorf,
Switzerland

KIJIMA *ET AL.* REPLY—We admit that we have misinterpreted the electron spin resonance (ESR) spectra given in Fig. 2 of our paper. It is true that these spectra are indicative of a random orientation of the adsorbed Cu(II) complexes rather than a spatial organization, as pointed out by Motschi. This indication, however, appears impossible in view of the stereochemistry of hydrated Cu(II) ions in Cu(II)-montmorillonite¹. By using X-ray diffraction and scanning electron microscopy, we have re-examined the orientation of silicate layers in the film of CDen-Cu-montmorillonite used for ESR measurements. This re-examination leads us to conclude that the ESR spectra in Fig. 2 should be for a randomly oriented sample, not for an oriented film.

In considering the arrangement of intercalated CDen, ESR observations were used to justify two assumptions: (1) that the interlayer Cu(II) ion forms a coordination group [Cu(II) (*en*) (H₂O)₂]²⁺ with a square configuration and (2) that this square plane is perpendicular to the silicate layers. The correct data support assumption (1), but are inefficient in proving the other. However, it does not mean that the structure model proposed is invalidated. This is because it seems reasonable to adopt the second assumption, and because the structure model was fundamentally deduced from the interpretation of data for the interlayer spacing

and chemical composition of CDen-Cu-montmorillonite. Thus, our conclusions about the lipid bilayer orientation remain unchanged.

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¹ Clementz, D. M., Pinnavaia, T. J. & Mortland, M. M. *J. chem. Phys.* **77**, 196 (1973)

Matters Arising

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¹ Kijima, T., Tanaka, J., Goto, M. & Matsui, Y. *Nature* **310**, 45-47 (1985)



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CAMBRIDGE
MRC Laboratory of
Molecular Biology
POST-DOCTORAL
SCIENTIST

Applications are invited from post-doctoral scientists to work with Dr. Tom Creighton on genetic engineering of a small protein for structural and folding studies. Experience of genetic manipulation techniques and protein chemistry will be advantageous but not essential.

The post is available for 3 years and the salary will be in the range £3,920 — £14,925 pa.

Applications — including a full CV, a short statement of relevant experience, and the names of two professional referees — should be sent by (8th August 1985) quoting reference BL/8 to: The Assistant Director (Administration), MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH. (7130)A

UNIVERSITY OF
LIVERPOOL
DEPARTMENT OF BOTANY
Applications are invited for an
SERC postdoctoral
SENIOR RESEARCH
ASSISTANT

tenable for twelve months at a salary of £7,520 per annum.

The project is an investigation of the ionic relations of the green alga *Dunaliella*. A knowledge of the use of radio-isotopes and the techniques of electro physiology would be helpful.

Informal enquiries may be made to Dr. J.C. Collins, Tel: 051-709 6022 Ext: 2400.

Applications, together with the names of three referees, should be received as soon as possible by The Registrar, The University, P.O. Box 147, Liverpool, L69 3BX from whom further particulars may be obtained.

Quote Ref: RV/153/N. (7141)A

THE MIDDLESEX
HOSPITAL
MEDICAL SCHOOL

RESEARCH WORKER

Research worker required to participate in project on renal disease (hyperfiltration syndromes). The ability and dedication to carry out high precision radioimmunoassays (RIA) will be essential and familiarity with RIA and computer handling of data, an advantage. The project is suitable for work towards a higher degree. Salary up to £8,753, according to age and experience. The position is available for 3 years (first six months are a probationary period).

Applications with full cv and names and addresses of 2 referees should be sent not later than 31 July 1985 to: The Administrative Officer, The Middlesex Hospital Medical School, Cleveland Street, London W1P 7PN. (7151)A

THE UNITED MEDICAL
AND DENTAL SCHOOLS

Guy's Campus — Paediatric
Research Unit

Human Genetic Diseases

RESEARCH ASSISTANT

required to join a project investigating the basic defect in Cystic Fibrosis. The post will involve a biological and biochemical analysis of specific epithelial cell types. Experience of cell culture techniques is essential.

The post will be for a period of two years. Initial salary up to £6,600 pa plus £1,233 pa London Allowance.

Applications, including full Curriculum Vitae and names of two referees, to the Administrative Assistant, The Prince Philip Research Laboratories, Guy's Tower, London SE1 9RT. (7119)A

PARKE-DAVIS RESEARCH UNIT

CAMBRIDGE

DIRECTOR: Dr John Hughes

RESEARCH ASSOCIATE IN PHARMACOLOGY

The Unit is developing new generation drugs for the treatment of nervous and neurohormonal disorders. Our basic research interests centre on the function and mechanism of action of hormones and neurotransmitters at the behavioural, cellular and molecular levels with particular reference to peptides. The Unit is a unique example of academic and industrial collaboration aimed at solving problems of fundamental importance to human health.

Applications are invited from experienced research pharmacologists ideally with expertise in isolated tissue techniques. Applicants should have at least three years postgraduate research experience preferably within the pharmaceutical industry, or hold, or expect to obtain by 1st October, a Ph.D in a relevant pharmacological area.

The position will carry a starting salary in the range £9,600 - £11,000 depending on experience. Other benefits include relocation assistance where appropriate.

Applications should be accompanied by a full up-to-date c.v. describing experience and research interests, together with the names of two scientific references and sent to Dr. Chris Bruton, Parke Davis Research Unit, Addenbrookes Hospital Site, Hills Road, Cambridge CB2 2QB to arrive as soon as possible but in any event by 3rd August 1985.

(7175)A

WARNER
LAMBERT



UNIVERSITY C
FACUL

Medical Science and Clinical Appointments

Applications are invited for the following appointments in the Faculty of Medicine

Anatomy

- a Assistant/Associate Professor in Gross Anatomy — 1 pc
Candidates should have teaching experience in Topographic Anatomy. Current and active research experience may be in the areas of Neuroanatomy, Histology, Growth and development or Gross Anatomy

Psychiatry

- a Associate Professor — 1 pc
Candidates should have experience in teaching and/or research in clinical psychiatry, preference will be given to suitable candidates who speak Arabic
b Assistant Professor — 1 pc

Medicine

- a Associate/Full Professor in Dermatology — 1 pc

Paediatrics

- a Associate Professor — 2 pos
in Paediatric endocrinology Allergy or gastroenterology

Physiology

- a. Associate/Full Professor — 2 pos
Preference will be given to general systemic physiologists with balanced experience in teaching and research.

Pharmacology

- a. Associate/Full Professor in Toxicology — 1 pc
b. Associate Professor in Clinical Pharmacology — 1 pc
The applicant for the post of Toxicology should have experience in teaching and toxicological services, such as therapeutic drug monitoring and toxicological screening.

Surgery

- a Professor ENT — 1 pc
b Associate Professor ENT — 1 pc
c Associate Professor in Anaesthesia — 1 pc
d Associate Professor in Orthopaedic Surgery — 1 pc
Candidates for surgical posts should have at least 5 years clinical and teaching experience. They should also possess a Ph.D. or high professional medical qualifications (e.g. F.R.C.S.) in their respective fields.

Diagnostic Radiology

- a. Professor — 1 pc
b. Associate Professor — 2 pos
c. Assistant Professor — 1 pc
Experience in one or more of the following areas is needed, Body-CT, Orthopedic Radiology, Paediatric Radiology, GI and GU Radiology, and US Interventional Radiology.

Nuclear Medicine

- a Full Professor — 1 pc
b Assistant Professor — 2 pos
Experience and ability to teach are highly required. Candidates must have been involved in undergraduate and postgraduate teaching and basic and clinical research. Knowledge of computers applied to medicine is desirable.

Organ Transplantation

- a Assistant/Associate Professor in Transplantation Surgery — 2 pos
Applicants should have a background of general, vascular or urological surgery, possess the Fellowship of the Royal College or be certified by a recognised Board of Surgery or their equivalent. They should have 2 to 3 years of training in any area of clinical organ transplantation. For the Associate Professor another additional 3 to 4 years of practice in clinical transplantation surgery are required.
b. Assistant Professor in Experimental Surgery — 1 pc
Applicants should have a background in surgery or immuno-biology, possess a surgical Fellowship or a Ph.D. or equivalent and have 2 to 3 years of experience in surgical research preferably in an area related to Organ Transplantation.
c. Assistant/Associate Professor in Clinical Bone Marrow Transplantation — 1 pc
Applicants should have a background in haematology, oncology or immunology, with Postgraduate Fellowship, Membership or Certification in the specialty and have 2 to 3 years of training in Clinical Bone Marrow Transplantation. For the position of Associate Professor 3 to 4 years of experience in clinical Bone Marrow Transplantation will be preferred.
d Assistant/Associate Professor in Transplant Intensive Care — 1 pc
Applicants should have a background in Anaesthesia, Respiratory Care or Metabolism, possess the appropriate Fellowship, Membership or appropriate Board Certification or equivalent and have 3 to 4 years of experience in the field.

Biochemistry

- a. Associate Professor — 2 pos
b Assistant Professor — 1 pos

KUWAIT HEALTH SCIENCE CENTRE MEDICINE

Pathology

- Histopathology/Cytology
 - Full Professor — 1 post
 - Associate Professor — 1 post
 - Assistant Professor — 1 post
- Clinical Chemistry
 - Full Professor — 1 post
- Haematology
 - Associate Professor — 1 post

Microbiology

- Clinical Immunologist
 - Associate Professor — 1 post
 1. Candidate should be experienced in Clinical Immunology with a minimum of 3 years experience in the routine operation of a routine clinical immunology laboratory.
 2. Candidate should have a Ph.D./MD and Professional qualification e.g. M.R.C. Path or American Board or equivalent degree.
- Clinical Virologist
 - Professor/Associate Professor — 1 post
 1. Will be responsible for viral serology unit which mainly involves hepatitis and rubella serology. He will be responsible for cell culture laboratory, virus isolations and preparation of viral antigens and antiserum. Both jobs include teaching, research and clinical work.
 2. Clinical Virology experience. MD, Ph.D. MRCP or equivalent. Internationally known for work in clinical virology.

Virologist

- Associate Professor/Assistant Professor — 1 post
- Job description: Basic virology research, or molecular virology. Teaching undergraduates and postgraduates. Research in basic virology.
- Ph.D. with sound research experience

Community Medicine and Behavioural Sciences

- Associate Professor in Medical Psychology — 1 post
- Applicants should be able to demonstrate teaching and research competence. Preference will be given to candidates who have experience in teaching introductory and medical psychology courses to medical and allied health students, and who are interested in contributing to the development of postgraduate programmes.
- Associate/Full Professor in Epidemiology — 1 post
- Assistant/Associate Professor in Epidemiology — 1 post
- Applicant should be able to demonstrate teaching and research competence in the application of epidemiological methods to either etiological or health services development problems. The successful candidate is expected to take active part in undergraduate and postgraduate teaching, using field demonstration and small group teaching techniques. He would be encouraged to follow his own line of research in addition to assisting the Department, Faculty and Ministry staff in the design and conduct of quantitative studies.

Requirements for Appointment

Applicants should possess a Ph.D. or higher professional qualification, i.e. F.R.C.S., M.R.C.P. etc. in their respective speciality and have conducted and published research in their field. Professors should have 14 years' experience, 4 as an associate professor or its equivalent, i.e. senior lecturer or reader. Associate Professors should have 9 years' experience, 4 as an assistant professor or its equivalent, i.e. lecturer.

Conditions of Appointment

- Salaries: — Total monthly salaries will be within the following scales according to qualifications and experience (1 KD = £2.5, US\$3.2 approx.).
- Professors with clinical appointments = KD1210–KD1370 (8 increments).
- Professors medically qualified with medical science appointments = KD1140–KD1300 (8 increments).
- Professors non-medically qualified = KD1070–KD1230 (8 increments).
- Associate Professors with clinical appointments = KD989–KD1149 (8 increments).
- Associate Professors medically qualified with medical science appointments = KD932–KD1092 (8 increments).
- Associate Professors non-medically qualified = KD875–KD1035 (8 increments).
- Assistant Professors with clinical appointments = KD768–KD928 (8 increments).
- Assistant Professors medically qualified with medical science appointments = KD724–KD884 (8 increments).
- Assistant Professors non-medically qualified = KD680–KD840 (8 increments).
- Clinical Supplements: In addition to the above university salaries there will be monthly clinical supplement paid by the Ministry of Public Health for 10 months a year to medical school staff with clinical service commitments.
- These are:

Professor and Chairman	KD250,
Professor	KD200,
Associate Professor	KD150,
Assistant Professor	KD100.

Conference: A member is entitled to attend one academic conference a year which would be subject to the University rules and regulations.

Gratuity: There is a gratuity of one month basic salary for each year employed payable on termination of contract.

Housing: Suitable furnished, air-conditioned accommodation, electricity and water free of charge.

Medical care: Free, comprehensive treatment is available in Kuwait under the State Health Service.

Travel: Air tickets are provided from the country of recruitment for the appointee, spouse and up to 3 dependant children under 20 years. Hereafter, return air tickets are issued annually to the country of citizenship or permanent residence. On termination of contract, air tickets are provided to the country of recruitment. A baggage and freight allowance is also provided.

Vacation: 60 days paid annual leave and various national holidays.

Education: This is provided free in State Schools where the instruction is in Arabic. Staff who have to send their children to non-Arabic schools in Kuwait will have the tuition fees up to a maximum of 3 children met by the University.

Taxation: There is no income tax in Kuwait. Currency is transferable without restriction.

Method of Application

Curriculum vitae in duplicate which should include the names of 3 referees, personal particulars, qualifications with dates, career history, teaching experience, research accomplishments and where appropriate clinical experience should be sent to the Director of Planning and Academic Staff Recruitment, Faculty of Medicine, University of Kuwait, Health Science Centre, P.O. Box 24923, Safat, Kuwait, to arrive no later than 30th October, 1985.

(W2037)A

Astrophysics Group Leader

A vacancy exists at the Rutherford Appleton Laboratory for the Astrophysics Group Leader at Grade 6 (Senior Principal Scientific Officer) level. The work of the Group includes support of the University Community and research in the fields of x-ray, ultraviolet and infra-red astronomy and in solar physics

The duties of the Group Leader include: interface to the University Community involved in space projects supported by the Laboratory; the direction and co-ordination of the research programme in astronomy within the Laboratory; the management of the Group comprising of approximately 20 staff; provision of scientific support to instrument development and satellite project activities. He or she will also take responsibility for maintaining close links with the UK astronomical community, and with international agencies such as the European Space Agency and NASA

Within the Laboratory the Group Leader will be a member of a senior management team responsible for the implementation of the programme of the Astronomy, Space and Radio Board of the SERC at RAL.

QUALIFICATIONS AND EXPERIENCE:

The Group Leader will be expected to have an established reputation in a leading research team, for example as evidenced by a significant publication record, and will preferably have practical experience in space science or other relevant work

The appointment may be held on a permanent basis, or for a fixed term period of up to 5 years.

Salary range £17000 - £22926 per annum

The Rutherford Appleton Laboratory offers excellent working conditions and benefits including a good transport service and generous holidays. This is a pensionable appointment.

Contact Recruitment Office, Rutherford Appleton Laboratory, Science & Engineering Research Council, Chilton, Didcot, Oxon, OX11 0QX. Telephone Abingdon (0235) 445435 quoting Ref VN 359.

Closing date for applications: 9th August 1985.

(7178)A



The European Molecular Biology Laboratory, an international research organization situated in Heidelberg, West Germany, invites applicants for a position as

TECHNICAL ASSISTANT

The successful candidate will join a research group in our Cell Biology Programme and will carry out work on protein secretion, protein sorting and post-translational modifications of proteins

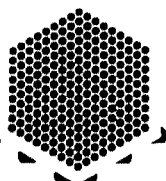
Applicants should be familiar with procedures used in tissue culture and monoclonal antibody production or protein purification and HPLC or molecular biology/molecular genetics. Knowledge of English or German as a working language is essential

An above-average salary will be offered to the successful candidate. Certain allowances are payable in addition, depending on personal circumstances. An initial contract of 3 years' duration, which can be renewed, will be offered

Please write briefly for an application form,

quoting reference
85/30 to
EMBL, Personnel
Section,
Postfach 10.2209,
D-6900
Heidelberg

(W2040)A



EMBL

UNIVERSITY OF ST ANDREWS

DEPARTMENT OF PHYSIOLOGY
AND PHARMACOLOGY

Applications are invited for a post of

POSTDOCTORAL RESEARCH ASSISTANT

funded by the British Heart Foundation for a fixed period of two years from 1st October, 1985. The research work, with Professor Lamb's group, will include studies of the regulation of mammalian Na, K-ATPase specific mRNA in cultured and collagenase dispersed cell systems. Applicants should have experience with recombinant DNA and tissue culture techniques. Experience in the isolation of ventricular myocytes would also be advantageous.

Starting salary within range £7,520 — £8,920 per annum on 1A scale, according to age, qualifications and experience.

Applications (two copies preferably in typescript) with the names of two referees should be lodged by 31 July, 1985 with the Establishments Officer, The University, College Gate, St Andrews, Fife, KY16 9AJ.

(7121)A

MOLECULAR BIOLOGIST

New, modern research unit emphasizing protein chemistry is seeking an investigator interested in the structure and regulation of eukaryotic genes using recombinant DNA technology. Ph.D. or equivalent and relevant postdoctoral experience required. Available Fall 1985, this position is of indefinite duration dependent upon performance. Salary and rank will be commensurate with experience. If interested, send a current CV and a statement of research interests and goals to.

Sally C. Gay
Personnel Director
Shriners Hospital for
Crippled Children
3101 SW Sam Jackson
Park Road
Portland OR 97201

Equal Opportunity Employer.
(NW38)A

MOLECULAR BIOLOGIST

Tenure-track position at the assistant professor level for a molecular biologist. Applicant must have at least 2 years postdoctoral experience and demonstrated accomplishment in eukaryote gene expression studies. Expertise in recombinant DNA technology essential. Ph.D. or M.D. Send curriculum vitae, a statement of research interest, and the names and addresses of 3 references to: Dr. Ralph Weichseleum, Professor & Chairman, Dept. of Radiation Oncology, Biological Sciences Division, The University of Chicago, Box 440, 5841 S. Maryland Ave., Chicago, IL 60637. An Affirmative Action Equal Opportunity Employer.

(NW1874)A

UNIVERSITY OF OXFORD

Laboratory of Molecular
Biophysics

Protein Engineering of
Antibody Combining Sites

AN SERC SUPPORTED
POSTDOCTORAL
POSITION

is available from October 1st 1985 for a molecular biologist to work with Dr A R Rees on the engineering of anti-protein monoclonal antibody genes via site-directed mutagenesis. The program is multidisciplinary in approach and combines X-ray crystallography, computer graphic modelling and recombinant DNA techniques.

Applicants should have experience in cloning methods. The appointment will be on the Research Assistant 1A Scale, starting in the range £7,520 — £8,450 and will be for three years.

Applications in writing, giving the names of three referees should be sent to: Professor Sir David Phillips, Laboratory of Molecular Biophysics, The Rex Richards Building, South Parks Road, Oxford OX1 3QU

(7129)A

TECHNICAL ASSISTANT

quired to work in the Biological Electron Microscope Unit, Queen Mary College, London for one year the first instance. A person with E experience would be preferred at this is not essential as training n be given. The salary could be up £8,000.00 pa (including London eighting). The appointment to mmmence 1st October 1985

For further information please nctact— J B Kirkham,, Biological M Unit, Queen Mary College, le End Road, London E1 4NS. (7149)A

ROYAL POSTGRADUATE MEDICAL SCHOOL (Hammersmith Hospital)

DEPARTMENT OF MEDICINE TECHNICIAN/ JUNIOR TECHNICIAN

Technician is required to join the Cardiovascular Diseases Unit to ke part in a wide range of cardiac agnostic techniques suvh as rdiac Catheterisation, Echocardi- graphy etc. and to help in the main- nance of equipment. A knowledge basic electronics would be an Advantage

Applicants will be considered cording to qualification and ex- rience, with salary on the range ,795 to £8,177 plus £1,042 London llowance for applicants with HNC/ EC level 5, or graduates having a egree plus one year relevant perience.

Graduates without relevant experi ice would be considered for ppointment as a Junior Technician, ith promotion to Technician after ie year of satisfactory work

Application forms and further par- ulars may be obtained from the rsonnel Office, Royal Postgradu- e Medical School, 150 Ducane Rd, /12 0HS quoting ref: 2/JL/N

Closing Date: 1 August

(7127)A

LECTURESHIP IN LIFE SCIENCES

Applications are invited for the above post. The successful applicant will have particular responsibility for a postgraduate course in Fisheries Biology, and should therefore have expertise in some area of fish biology.

The post is tenable from 1st January, 1986

Starting salary in the range £8,000 to £15,000. USS

Closing date for applications 28th August, 1985

Further particulars from The Registrar.

The University of Buckingham, Buckingham MK18 1EG

(7155)A

The
University of
Buckingham

Molecular Biologist and Cell Culture Scientist

Post Doctoral research in drug metabolism

Smith Kline and French Research have an exciting Post Doctoral Research Fellowship in Drug Metabolism and Pharmacokinetics to investigate fundamental aspects of the molecular biology of cellular toxicity and its relationship with the expression of drug-metabolising enzymes.

The work will be concerned with the study of factors affecting the expression of a defined drug metabolising system in vivo and in cell culture. Enzyme purification, antibody production, molecular cloning and possibly gene sequencing techniques will be involved.

Excellent academic and practical skills and the ability to apply novel, inventive approaches to problem solving are required. The post should appeal to molecular biologists and specialists in cell culture techniques who wish to apply their skills in a new field as well as to those who have previous experience in drug metabolism. Publication of scientific papers will be encouraged.

We offer competitive salaries and benefits for this 2/3 year fixed term appointment.

Please send a cv outlining your qualifications and experience to the Personnel Officer quoting reference number N/134/JDW, Smith Kline & French Research Limited, Research Institute, The Frythe, Welwyn, Herts AL6 9AR. (7177)A

UNIVERSITY OF GLASGOW

DEPARTMENT OF VETERINARY PATHOLOGY POSTGRADUATE RESEARCH ASSISTANT IN MOLECULAR BIOLOGY

Applications are invited from post-graduate scientists with a degree in molecular biology, microbiology, or a related discipline to work on an AFRC funded project to develop retrovirus vectors for gene insertion into embryos.

The successful candidate will have familiarity with, and an interest in current techniques in recombinant DNA technology.

In the first instance this post will be funded for 2 years from October 1985 on the 1B Scale

Applications, including a curriculum vitae and the names of two referees should be sent to Dr. D Onions, from whom further information may be obtained, at the Department of Veterinary Pathology, University of Glasgow Veterinary School, Bearsden Road, Bearsden, Glasgow G61 1QH. (7140)A

THE MEDICAL COLLEGE OF ST. BATHOLOMEW'S HOSPITAL (University of London)

Department of Biochemistry

Applications are invited for a post of

GRADUATE RESEARCH ASSISTANT

To work on electrostatic effects in enzyme catalysis using a wide range of kinetic, spectroscopic and protein chemical techniques applied to the cysteine proteinases, a group of homologous enzymes that provide natural structural variation in mechanistically relevant regions of the molecule. Applicants should have a First or Upper Second Class Honours degree in Biochemistry or Chemistry and would be expected to register for a higher degree. The post is funded by the SERC for 3 years from 1st October, 1985. Salary £7,833 — £8,753 including L.W. plus USS if required.

Applications with full curriculum vitae and names of two referees should be sent as soon as possible to Dr. K. Brickleyhurst, Department of Biochemistry, Medical College of St. Bartholomew's Hospital, Charterhouse Square, London, EC1M 6BQ. (7160)A



**INVERESK
RESEARCH
INTERNATIONAL**

DEPUTY HEAD — BIOTECHNOLOGY

Inveresk Research International Ltd. is one of the UK's largest independent contract research organisations. One of its main areas of activity is in contract Biotechnology. This is concerned with the development of novel product and processes in the health care, agricultural and food sectors. This group is in a phase of rapid expansion and now seeks to recruit a Deputy Head.

Research activities include extensive genetic engineering studies with microbial and mammalian vector systems, mouse and human monoclonal antibody developments, the design of novel immunodiagnoses, as well as traditional biochemical, genetical and enzymological studies. The appointee will, therefore, be expected to make a major contribution to the management of these areas and to provide the highest calibre of intellectual input to problem solving and innovative research in a highly competitive market place.

Applicants should have a sound knowledge of Biochemistry, Molecular Biology and Genetics, as well as a minimum of 10 years postgraduate experience. Proven ability in the management of a research team would be a prerequisite.

Salary for this appointment will be dependent upon qualifications and experience, and will reflect the importance of this position. In addition we offer a range of modern staff benefits including an excellent Pension & Life Assurance Plan, free private health care, as well as assistance with relocation expenses, where appropriate.

Full details in writing should be forwarded, in confidence, to the Personnel Department, Inveresk Research International Limited, Inveresk Gate, Musselburgh EH21 7UB, quoting reference 2544.

(7152)A

RESEARCH ASSOCIATE OR POSTDOCTORAL POSITIONS

Three positions available immediately for persons with training in molecular biology and nucleic acid biochemistry to work on the development of infectious vaccinia virus recombinant vaccines for viral diseases of cattle and to work on the expression of cloned cDNAs in prokaryotic and eukaryotic systems.

Salary: £17,000 to £25,000 depending on qualifications. Positions are funded for at least two years.

Send curriculum vitae and three letters of reference to: T. Yilma, Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA 99164-7040; phone (509) 335-6042. Washington State University is an affirmative action/equal opportunity employer. Women and candidates from minority groups are encouraged to identify themselves as such.

(NW42)A

Postdoctoral Positions in Plant Molecular Biology

Two positions are available to study molecular biology of maize in an interdisciplinary project at the University of Minnesota. Research areas include transcriptional regulation of gene expression especially related to rRNA, zein or aspartate family pathway genes, and mitochondrial gene organization and expression.

Applicants must have completed the requirements for a Ph.D. by the time of appointment and should send a letter of intent, curriculum vitae, and statement of research experience and have 3 letters of recommendation sent to: Dr. B. Gengenbach, Dept. Agronomy and Plant Genetics, University of Minnesota, St. Paul MN 55108 by August 31, 1985.

The University of Minnesota is an Equal Opportunity Educator and Employer and specifically invites and encourages applications from women and minorities.

(NW48)A

THE DEPARTMENT OF PEDIATRICS University of California, San Diego

is recruiting for an

ASSISTANT PROFESSOR

In Residence in Neonatal/Perinatal Medicine

Position includes clinical supervision and teaching of housestaff and medical students, with unlimited opportunity for newborn research. Applicants must be board certified pediatrician or board qualified, with post-graduate training in neonatal/perinatology; licensed or licensable to practice medicine in the State of California; experience in teaching and clinical service; with research background. Salary commensurate with skills, knowledge, and University of California policy.

Candidates apply by August 15, 1985 with C.V. to Ronald Coen, M.D., Associate Professor of Pediatrics, Department of Pediatrics, UCSD Medical Center H-638-A San Diego, CA 92103. EEO/AA.

(NW43)A

BEATSON INSTITUTE FOR CANCER RESEARCH RESEARCH TECHNICIAN/ RESEARCH OFFICER

A new position for a research technician or research officer has arisen to work in a group studying the molecular control mechanisms regulating cell differentiation in the red blood cell system and how this becomes aberrant in certain leukaemias.

Applicants should have a good Honours degree in biochemistry, molecular biology or a related subject and/or technical experience in one of these areas. The position has long term funding and is an attractive position for someone highly motivated for a career in research. The position which is available from 1st October 1985 will be on MRC scales for research technicians or research officers (currently £5,952 — £8,096 or £6,483 — £8,492 respectively).

Interested applicants should send a full CV and the names of two academic references to Dr Paul Harrison, The Beatson Institute for Cancer Research, Garscube Estate, Switchback Road, Bearsden, Glasgow G61 1BD (Telephone: 041 — 942 9361).

(7136)A

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE (University of London) GRADUATE RESEARCH STUDENT

Applications are invited from students expecting to graduate in 1985 with at least 2:1 in Pharmacology, Physiology or related subject. The successful applicant will need to be confident in the use of quantitative approach. The research (supported by an Action Research project grant for a research assistant) involves investigation of the electro-physiology of neuromuscular transmission in myasthenic disorder using modern microelectrode techniques. The study is to be undertaken jointly by the Department of Pharmacology and Neurological Science during three year period, starting October 1985, and the applicant will be registered for a PhD.

Full curriculum vitae including names and address of two referees should be sent immediately to the School Office, RFHSM, Rowland Hill Street, London, NW3 2PF — please quote reference DW/GR. For further details telephone 794-0500 Extn 4330 or 4363. Closing date 2 August 1985.

(7132)A

THE DEPARTMENT OF PHYSIOLOGY University of Manitoba

invites applications from young investigators in the field
of

CARDIOVASCULAR PHYSIOLOGY

to be appointed at the level of Assistant Professor

The successful candidates will be expected to compete for local and national scholarship awards and develop their independent research programs. Applicants with backgrounds in membrane transport, electrophysiology, molecular biology or hemodynamics are encouraged to submit their curriculum vitae, an outline of their research interests as well as to arrange for three letters of reference to be sent to Dr. Henry G. Friesen, Professor and Head, Department of Physiology, Faculty of Medicine, University of Manitoba, Winnipeg, Manitoba, R3E 0W3 Canada. Deadline for application is 2 September 1985. The University of Manitoba is an Equal Opportunity/Affirmative Employer, but in accordance with Canadian Immigration, priority will be given to Canadian citizens and permanent residents of Canada. (NW12)A

PHYSICAL BIOCHEMISTS/MEMBRANE AND CELL BIOLOGISTS

The Thrombosis/Hematology Research Program of the Oklahoma Medical Research Foundation is seeking highly qualified M.D.'s and Ph.D.'s to participate in research related to the interactions of the serum complement, coagulation and fibrinolytic systems on membrane surfaces. Prior training and experience in protein chemistry, membrane biology, and/or the application of physical methods (e.g., *in vivo* fluorescence spectroscopy) to cell biology desired.

Suitable candidates will be expected to conduct full-time laboratory research in close interaction with the Thrombosis/Hematology Research Program. Funded positions available at all levels (postdoctoral and more senior positions) depending on experience.

Mail vitae and description of research interests to Peter J. Sims, M.D., Ph.D., Associate Member, Oklahoma Medical Research Foundation, 825 Northeast Thirteenth Street, Oklahoma City, Oklahoma 73104.

An equal opportunity, affirmative action employer. (NW41)A

(Re-advertisement) EAST MALLING RESEARCH STATION CELL PHYSIOLOGIST

Research post, funded for 33 months, available within group investigating somaclonal variation in strawberries. The successful applicant will be responsible for developing methods for strawberry protoplast culture, design and application of appropriate selection procedures and regeneration of plants.

Qualifications: good honours degree preferably with PhD or at least two years postgraduate research in an appropriate biological discipline. Experience of protoplasts and tissue culture essential, biochemical training useful.

Salary in scale £7,788 — £10,541 per annum.

Further details from Secretary, East Malling Research Station, Maidstone, Kent. ME19 6BJ

East Malling Research Station is an equal opportunity employer. (7144)A

UNIVERSITY OF GLASGOW DEPARTMENT OF VETERINARY PATHOLOGY POSTDOCTORAL RESEARCH ASSISTANT IN VIROLOGY

Applications are invited from post-doctoral scientists to work on a project to develop novel vaccines against equine herpes virus 1 using recombinant DNA techniques.

The successful candidate will have had extensive experience of current techniques in recombinant DNA technology, preferably with a background in virology. This post will be funded for a period of 3 years by the Horserace Betting Levy Board starting in October 1985. The salary will be on the 1A Scale.

Applications, including a curriculum vitae and the names of two referees, should be sent to Dr D. Onions, from whom further information may be obtained, at the Department of Veterinary Pathology, University of Glasgow Veterinary School, Bearsden Road, Bearsden, Glasgow G61 1QH. (7139)A

RU Leiden

The Center for Bio-Pharmaceutical Sciences has a vacancy for a

full professor of pharmacognosy, in particular plant cell biotechnology

vacancy nr 5-106/2407

The Division of Pharmacognosy of the Center conducts research on the phytochemical, analytical and enzymological aspects of the biosynthesis of secondary plant metabolites of a primarily pharmaceutical importance.

This research is intended to provide a basis of the development of new drug manufacturing techniques by means of plant cell biotechnology

The Center for Bio-Pharmaceutical Sciences is a new part of the Faculty of Mathematics and Natural Sciences.

Its primary objective is to conduct multidisciplinary research into the production of drugs, and their disposition and effects in man and animal. In close relation to its research, the Center will also offer a 3 year undergraduate program (M. Sc. Level) as well as a 4 year graduate program (Ph. D. level).

In addition to the Division of Pharmacognosy, the Center contains Divisions of Analytical Chemistry, Biopharmaceutics, Pharmaceutical Technology, Pharmacology, Medicinal Chemistry and Toxicology. The Division of Pharmacognosy is a partner in the Plant Cell Biotechnology Project Group (Biotechnology Delft-Leiden, BDL), together with the Departments of Plant Molecular Biology (Leiden University) and Biochemical Engineering and Applied Enzymology (Delft University of Technology).

The profile of the Center has been specified in a brochure which may be obtained from the Secretary of the Selection Advisory Committee Mr O. H. Postma, P.O. Box 9502, 2300 RA Leiden, The Netherlands. (Phone: 071-148333 extension 4219/4788)

Candidates should have a vast experience relevant to the research program of the Division of Pharmacognosy. They are expected to be able to stimulate research collaboration with other Divisions of the Center as well as with outside partners, including industry.

Proficiency in management of multidisciplinary research is an asset. In addition, candidates should be prepared to engage in the design and teaching of undergraduate and graduate courses. Candidates should also be willing to learn Dutch.

Candidates are invited to send a written application, with full C.V., to the Secretary of the Selection Advisory Committee, Center for Bio-Pharmaceutical Sciences, Gorlaeus Lab., P.O. Box 9502, 2500 RA Leiden, The Netherlands within two months after the publication of this advertisement.

Further information may be obtained from the Committee Chairman, Prof. dr K. R. Lebbenga, Dept of Plant Molecular Biology, Nonnensteeg 3, 2311 VJ Leiden, The Netherlands. (phone: 071-148333 ext 6664 or 6665).

(W2033)A

KUWAIT

University of Kuwait Health Science Centre Faculty of Medicine

Applications are invited for the posts of Chief Technician in the Department of Surgery, Paediatrics, Community Medicine and Behavioural Science, Microbiology (Virology and Immunology). Candidates should have experience as a clinical laboratory technician and hold the FIMLS, or equivalent qualification with 15 years' experience including training. The successful candidates' duties will be of a multi-discipline nature and will be both in laboratory and in the field.

Salary will be in the range KD450 - 512 per month, (KD1 = £2.5, US\$ 3.2 approx.) There is no income tax in Kuwait and currency is transferable without restriction. Free furnished, air-conditioned accommodation is provided, and electricity and water supplied free of charge. Sixty days paid annual leave for each completed year of employment, and annual economy class return air tickets to the country of citizenship or permanent residence are provided for the appointee, spouse and three dependent children. Free medical treatment is available under the State Health Service.

Applications should be submitted to: The Director of Planning and Academic Staff Recruitment, Faculty of Medicine, University of Kuwait Health Science Centre, P.O. Box 24923 Safat, Kuwait, with detailed curriculum vitae in duplicate, recent passport photograph, and the name of three referees, to arrive not later than 30th October, 1985. (W2036)A

MOLECULAR BIOLOGY OF ONCOGENES

IMMUNEX CORPORATION is a Seattle-based biotechnology corporation specializing in the commercial development of lymphokines and immune response modifiers. We wish to expand our group investigating the molecular biology of oncogenes and cloned lymphokine/lymphokine receptor genes. The projects are aimed at increasing our understanding of cell growth regulation in the lymphoid and hematopoietic systems, and require a collaborative approach in a stimulating multidisciplinary environment.

Two positions are available, one for a recent Ph D. graduate, the other requiring post-doctoral experience. The company offers competitive salaries, an equity participation program and a full benefits package.

Application, including CV, list of publications and names and addresses of three referees should be sent to the **Personnel Officer, Immunex Corporation, 51 University Street, Seattle, WA 98101**. Immunex Corporation is an equal opportunity employer.

(NW46)A

IMMUNEX

MEDICAL RESEARCH COUNCIL, Cell Biophysics Unit King's College London

Applications are invited for three three-year post-doctoral appointments:

1. in neurobiology, to work on development in the vertebrate visual system, studying interactions between neurones and glial tissues leading to pattern formation *in vivo*. Experience of cell biological and immunological techniques would be advantageous, (2597)

2. to work in collaboration with muscle and cell motility research groups on the cloning, expression and site-directed mutagenesis of actin. Experience with recombinant DNA techniques would be essential, (2560)

3. on the molecular mechanism of muscle contraction, including the use of fluorescent probes. A background in physiology or biochemistry would be appropriate; (5262)

The appointments will be on the Council's non-clinical salary scale, £7,520 — £15,925 plus £1,233 L.W. and enquiries may be made of Dr J.H. Scholes for position 1 and of Professor R.M. Simmons for positions 2 and 3, (01-836-8851). Applications (including full cv and the names and addresses of two referees, and mentioning which post) should be sent, by 22 August 1985, to: Mr D.M. Drummie, MRC Cell Biophysics Unit, King's College London, 26-29 Drury Lane, London WC2B 5RL. (7135)A

UNIVERSITY OF GLASGOW

DEPARTMENT OF VETERINARY
PATHOLOGY

POSTDOCTORAL RESEARCH ASSISTANT IN MOLECULAR BIOLOGY

Applications are invited from post-doctoral scientists to work on an AFRC funded project to develop retrovirus vectors for gene insertion into embryos.

The successful candidate will have had extensive experience of current techniques in recombinant DNA technology and preferably had experience of work in embryology or virus vectors.

In the first instance this post will be funded for 2 years from October 1985 on the 1A Scale.

Applications, including a curriculum vitae and the names of two referees should be sent to Dr D. Onions, from whom further information may be obtained, at the Department of Veterinary Pathology, University of Glasgow Veterinary School, Bearsden Road, Bearsden, Glasgow G61 1QH. (7137)A

THE MIDDLESEX HOSPITAL

MEDICAL SCHOOL
(University of London)

DEPARTMENT OF MEDICINE RESEARCH ASSISTANT

Applications are invited from science graduates to work on a project investigating the metabolism of vitamin D in man. Postgraduate experience will be an advantage. The research involves the development and clinical application of radioimmunoassays for the metabolites of vitamin D as well as *in vitro* studies of the mode of action of the vitamin. The appointment, funded by the MRC, is tenable until December 1986. Salary up to £10,153 according to age and experience.

Applications, together with telephone numbers and the names of 2 referees, should be sent to: Professor J. L. H. O'Riordan, Department of Medicine, The Middlesex Hospital, Mortimer Street, London W1N 8AA. (7171)A

UNIVERSITY OF EXETER

DEPARTMENT OF BIOLOGICAL
SCIENCES

POST-DOCTORAL RESEARCH ASSISTANT

Applications are invited for the above SERC post from suitably qualified Biochemists or Biologists. Chemists, for a period of 3 years commencing 1 October 1985, or as soon as possible thereafter.

The successful applicant will study the mode of entry of insect steroid hormones (ecdysteroids) into target cells. The project will involve the application of a wide range of biochemical techniques (cell culture, HPLC, affinity chromatography etc) and the chemical synthesis of hormone derivatives.

The starting salary will be within the range £7,520 — £8,450 per annum dependent on age and experience.

Applications including a CV and the names of 2 referees should be sent to Dr L. Dinan, University of Exeter, Exeter, EX4 4QG, from whom further details are available. Closing date 14 August 1985.

(7118)A

UNIVERSITY OF BRITISH COLUMBIA

Department of Botany

Researchers:

POSTDOCTORAL LEVEL and RESEARCH ASSISTANT TECHNICIAN

The project involves the use of the *Agrobacterium tumefaciens* system to introduce foreign genes into plant tissue. Investigation of viral expression, specifically tobacco mosaic virus will also be involved. Techniques of molecular genetics and/or microbial techniques and/or plant tissue culture sought.

To apply, please send curriculum vitae, and names and addresses of referees to: Dr John McPherson, Dept of Botany, #3529 — 6270 University Boulevard, University of British Columbia, Vancouver V6 2B1. (NW47)A

MOLECULAR CARDIOLOGIST

A tenure-track faculty position is available to study the molecular and genetic basis of cardiac muscle development. Experience with recombinant DNA techniques is essential; experience with somatic cell genetics is desirable.

Send Curriculum Vitae, a brief description of research experience and interests, and the names of three references to: Dr Robert Roberts, Chief, Section of Cardiology, Department of Medicine, Baylor College of Medicine, 6535 Fannin St., M.S. F-905, Houston, Texas, 77030. Equal Opportunity Employer. (NW44)A

THE MIDDLESEX
HOSPITAL
MEDICAL SCHOOL
LONDON W1P 6DB
(University of London)

DEPARTMENT OF ANATOMY AND
BIOLOGY AS APPLIED TO
MEDICINE

POST-DOCTORAL
RESEARCH ASSISTANT

Applications are invited for the SRC supported post, tenable from October 1 1985 (or earlier) to work for 3 years on cell contact and movement using advanced fluorescence methods.

Written applications, including the names of two referees, should be sent to Professor D Gingell at the above address. (7126)A

ROYAL POSTGRADUATE
MEDICAL SCHOOL
(University of London)
PAEDIATRICS AND
NEONATAL MEDICINE

Applications are invited for the post

RESEARCH ASSISTANT
in the Jerry Lewis
Muscle Research Centre

Work on a project concerned with the application of recombinant DNA techniques in familial studies of neuromuscular disorders. The project will involve identification of suitable families and collection and processing of samples. Experience of recombinant DNA techniques is essential. The post is funded by the Muscular Dystrophy Association of America and is for one year in the first instance.

Applicants should be graduates with relevant work experience.

Salary will be between £5,316 and £7,520 plus London Allowance, starting point depending on experience.

Application forms may be obtained from the Personnel Office, Royal Postgraduate Medical School, quoting reference number 28/287/N.

Closing Date: 1 August 1985.
(7128)A

UNIVERSITY OF GLASGOW

DEPARTMENT OF VETERINARY
PATHOLOGY
POSTGRADUATE
RESEARCH ASSISTANT
IN VIROLOGY

Applications are invited from postgraduate scientists with degrees in virology, microbiology, or a related discipline, to work on a project aimed at developing novel vaccines to equine herpes virus 1 using recombinant DNA technology.

The successful candidate will have a familiarity with, and an interest in recombinant DNA techniques. Suitably qualified candidates may register for a PhD degree. This post is funded by the Horserace Betting Levy Board for 3 years starting in October 1985 and will be on the 1B salary scale.

Applications, including a curriculum vitae and the names of two referees, should be sent to Dr D Omions, from whom further information may be obtained, at the Department of Veterinary Pathology, University of Glasgow Veterinary School, Bearsden Road, Bearsden, Glasgow G61 1QH. (7138)A

THE SCHOOL OF
PHARMACY
University of London

TOXICOLOGY UNIT,
DEPARTMENT OF
PHARMACOLOGY
LECTURER IN
HISTOPATHOLOGY/
EXPERIMENTAL
PATHOLOGY

required to teach Histology and Comparative Pathology in the BSc course in Toxicology and Pharmacology. Applicants should have strong research interests involving Histology, Pathology or Quantitative Cytochemistry.

Salary in the range £7,520 — £14,925 plus £1,233 pa London Allowance. Further particulars from Dr G Cohen (01-837 7651)

Letters of applications with 2 copies of CV and names of 2 referees to: Personnel Officer, The School of Pharmacy, 29/39 Brunswick Square, London WC1N 1AX, by 31 August 1985, quoting reference GMC/N. (7150)A

INORGANIC CHEMISTRY

Applications are invited for the Chair of Inorganic Chemistry. The person appointed will guide teaching in organic chemistry — initiate research and supervise candidates for higher degrees, participate in the administration of the Department, including perhaps accepting the Headship of the Department, for periods. Inorganic research currently pursued in the Department lies in the fields of bio-organic co-ordination, macrocyclic and organometallic chemistries, and in homogeneous and heterogeneous catalysis.

Closing date 31 August 1985, late applications will be considered.

MICROBIOLOGY

Microbiologists with appropriate experience in one or more of the following fields are required for the posts of Associate Professor/Senior Lecturer and Lecturer in this expanding Department: Environmental Microbiology — Industrial Microbiology — Microbial Metabolism — Plant Virology — Animal Virology. The incumbents will be expected to be active research workers to participate in the teaching of the undergraduate and postgraduate courses given to students in the Faculty of Science and supervise research students. Duties are to be assumed as soon as possible.

Closing date 31 September 1985.

Salary: Professor R35,379; — R40,962; Associate Professor R34,071 — R36,684; Senior Lecturer R23,916 — R31,456; Lecturer R16,257 — £27,648. Benefits include an annual bonus • generous leave • pension • medical aid • remission of fees • sports facilities • clubs • housing subsidy, removal, travel and settling-in allowances (if eligible).

The policy of the University is not to discriminate on the grounds of sex, race, colour or national origin.

For further information contact Mr Geoff Henning, South African Universities Office, Chichester House, 278 High Holborn, London WC1V 7HE. (W2044)A



UNIVERSITY OF
THE WITWATERSRAND

KING'S COLLEGE LONDON
Department of Biophysics

HALF-TIME GRADE 5 TECHNICIAN (Permanent Post)

Enzymologist or physical chemist required for studies on the mechanism of chromatin transcription. The technician will have some responsibility for laboratory organisation. Salary pro-rata on the scale rising from £7,831 — £8,934 pa inclusive (under review).

Teaching Technician Grade 4 (Permanent Post)

Required to run the teaching laboratories. An interesting post concerned with a wide range of courses. Other duties include helping with the animal unit and assisting academic staff with research. Minimum qualification ONC or equivalent, plus at least 7 years relevant experience. Salary on scale rising from £7,356 — £8,274 pa inclusive. (Under review)

Grade 3 Technician (Short term Appointment)

Required for a project funded by the Leukaemia Research Fund to commence as soon as possible until 31st January 1988 on the cloning of genes for eukaryotic gene regulatory proteins. The technician will have some responsibility for organising the laboratory. Experience in recombinant DNA technology is required for this position. Salary on scale rising from £6,649 — £7,575 pa inclusive. (Under review)

Applications for these posts, which should include a C.V. and the names of two referees, should be sent to: Mr. D. Drummie, Administrative Assistant, Biophysics Department, King's College London, 26 Drury Lane, London WC2B 5RL.

Closing date: Friday 9th August 1985.

(7170)A

ONCOGENE SCIENCE INC.

Expanding staff at Long Island, NY research facilities due to advances in

Oncogene and growth factor research

to develop products for the early

detection -- monitoring -- treatment of human cancers.

— Positions Available —

DIRECTOR OF BIOCHEMISTRY

Responsible to direct and coordinate corporate biochemistry program. Ph.D. in Protein Chemistry — purification and sequencing; 8-10 years postdoctoral experience. Strong publication record highly desirable. Will contribute to development of diagnostic and pharmaceutical products.

PROJECT DIRECTOR

Responsible for formatting diagnostic tests for HTLV-III infection and oncogene activation. Test development, pilot and scale-up operations, standardization of appropriate QC procedures. Ph.D. in Immunochemistry. 5-7 years postdoctoral experience in immunoassay, format design, product development.

MOLECULAR BIOLOGIST

To participate in projects aimed at determining the role of oncogenes in human cancer using cDNA cloning in situ hybridization and bacterial expression of antigens. Ph.D. in Molecular Biology, although candidates with Masters degree and appropriate experience will be considered.

Be part of the excitement at OSI.

Send resume and personal data to Scientific Director, Oncogene Science Inc., 222 Station Plaza North, Suite 330. Mineola, NY 11501.

Competitive salary. Liberal benefits. Equity potential.

Equal Opportunity Employer.

(NW31)A

THE UNIVERSITY OF LEEDS

DEPARTMENT OF BIOCHEMISTRY

POSTDOCTORAL RESEARCH FELLOW

Applications are invited for the above post for work in the control of gene expression during Dictyostelium development involving the use of DNA Sequencing, site-specific mutagenesis and the transformation of chimaeric genes into responsive cells.

A good honours degree and a PhD or equivalent experience in a relevant subject are required. Previous experience in recombinant DNA

methodology would be an advantage

Post available from 1 October 1985 for a fixed period of up to three years

Salary within the range £7,520 — £8,920 (under review) on the 1A Range for Research and Analogous Staff, according to age, qualification and experience.

Informal enquiries may be made to Dr B D Hames (tel (0532) 431751 ext 7504).

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference number B3/65. Closing date for applications 8 August 1985. (7168)A

UNIVERSITY OF OXFORD

Action Research Professorship of Clinical Neurology

The electors intend to proceed to an election to the Action Research Professorship of Clinical Neurology with effect from 1 October 1987. The stipend of the professorship is at present £26,080, subject to review

Applications (ten copies, or one from overseas candidates), naming three referees but without testimonials, should be received not later than 16 September by the Registrar, University Offices, Wellington Square, Oxford, OX1 2JD, from whom further particulars may be obtained (7163)A

THE UNIVERSITY OF TASMANIA

PATHOLOGY: **POSTDOCTORAL FELLOW** **RESEARCH OFFICER** **(IMMUNOCHEMIST/ BIOCHEMIST)**

The Department of Pathology requires an immunochemist/biochemist to participate in a research programme on the local mechanism of tumour invasion and characterization of the enzymes involved. Applicants should hold a PhD degree and preferably have experience in enzyme biochemistry and immunological techniques. The position is funded by NH and MRC and the initial appointment will be until 3 December 1987. Salary will be within the range \$A22,614 — \$A23,934 pa. For further information about the post contact Professor H K Muller (tel 002-342866 ext 30)

Applications, which should include a cv with details of qualifications and experience, and names and addresses of 3 referees, should be sent to the Registrar, University of Tasmania, Box 252C, G PO Hobart Tasmania 7001, Australia, as soon as possible.

The University is an Equal Opportunity Employer. (W2038)A

UNIVERSITY OF MASSACHUSETTS MEDICAL SCHOOL

DEPARTMENT OF MOLECULAR GENETICS AND MICROBIOLOGY POST-DOCTORAL

RESEARCH FELLOW AND RESEARCH TECHNICIAN Yeast Molecular Genetics/ Secretion

(Ref: BC/133)

Applications are invited for the above positions to study expression of the *S. cerevisiae* dsRNA killer preprotoxin gene, secretion and maturation of its product, and its use for the secretion of foreign proteins in yeast. Post-doctoral applicants should have experience in recombinant DNA techniques and Technician applicants should have a degree or equivalent in biochemistry or a related discipline. Appropriate experience would be an advantage. Post-doctoral salary will be in accord with NIH guidelines and Technician salary will depend on experience and training, both in the range of £15,000 to £19,000.

Further details can be obtained from Dr D.J. Tipper, to whom applications, including a CV and three letters of recommendation, should be sent. Address until November 1st, 1985:

Department of Biochemistry,
University of Oxford,
South Parks Road,
Oxford OX1 3QU (UK)
Telephone 0865 56733 Ext 292

After November 1st, 1985:

Department of Mol. Genetics and Microbiology, U. Mass. Medical School, Worcester, MA 01605, USA
Telephone 617 856 2308. (7172)A

THE UNIVERSITY OF NEW ENGLAND

DEPARTMENT OF ANIMAL SCIENCE

RESEARCH APPOINTMENT

(Genetics of Host-Parasite Relationships)

Equal Opportunity is University Policy

Applications are invited for A Research Appointment (outside funded and subject to annual renewal) in a cooperative research programme between UNE and the CSIRO Divisions of Animal Production and Animal Health. The programme, which was initiated in 1981, is primarily concerned with the genetics of the interaction between sheep and the nematode parasite *Haemonchus contortus*.

The successful applicant will have responsibility for aspects of an investigation into the mechanisms which underlie genetic resistance to *H. contortus* and will work primarily with Dr G D Gray, Professors J S F Barker and R A Leng (UNE) and Drs L R Piper and L F Le Jambre (CSIRO).

Applicants should have a higher degree in physiology, immunology, biochemistry or a related field and have an interest in parasite infections of ruminants.

Informal enquiries may be made directly to Dr Gray in the first instance ISD (61 67 73-2226).

Starting Date: As soon as possible
Salary: \$A18,650 — \$A21,955 depending on qualifications and experience
Position No: 510
Closing Date: 15th August 1985

Applications, including the names and addresses of three referees and stating the position number should be sent to the Staff Officer, University of New England, Armidale N.S.W. 2351, prior to the closing date. Applicants should forward a copy of this advertisement to their referees and ask them to send their reports direct to the Staff Officer, Mr B.G. Turner, before the above closing date. (W2039)A

KINGSTON POLYTECHNIC

FACULTY OF SCIENCE

PRINCIPAL LECTURER IN APPLIED PHYSICS

Applicants for this senior post should be able to demonstrate achievements in an area of Applied Physics in industry, research or education. Areas of particular interest are Applied Optics, Materials, Microelectronics, Instrumentation and Computer Applications, Radiation Science and Acoustics.

The person appointed will be expected to take leadership in the development of collaborative research and have a strong interest in higher education. The post is viewed both as a new blood appointment and as a career opportunity for those with experience.

Salary range £13,773 — (bar) £15,258 — £17,145 including London allowance. Further details and application forms from Personnel Officer, Kingston Polytechnic, Penrhyn Road, Kingston upon Thames KT1 2EE. Tel 01-549-1366 ext 287. Closing date 2 September 1985. (7125)

DURHAM UNIVERSITY

DEPARTMENT OF GEOLOGICAL SCIENCES

Applications are invited for a

SENIOR RESEARCH ASSISTANT

1. Geophysics, tenable for three years, for work on 'Inter-borehole seismic surveys for opencast coal exploration', funded by S.E.R.C. and run jointly by the Departments of Engineering and Geological Sciences. It is concerned with the development of down-hole seismic sources and receivers to determine the continuity of coal seams using

channel waves. Instrument development, fieldwork, data processing and interpretation will be involved. The successful applicant is likely to have a good degree in Physics or Engineering with a PhD or equivalent research experience in a relevant field.

Initial salary £7,520 — £8,920 (under review) on Scale 1A with USS.

Applications (3 copies), naming three academic referees, should be sent by 31 August 1985 to The Registrar, Science Laboratories, South Road, Durham, DH1 3LE, from whom further particulars may be obtained. (7154)A

THE QUEEN'S UNIVERSITY OF BELFAST

MOLECULAR BIOLOGISTS

Northern Ireland Centre for Genetic Engineering

Two Research Officer positions are tenable from 1 October 1985 in this newly established Centre, (Director: Professor S J Martin, Department of Biochemistry) which will provide service/training facilities for the wide range of research projects are underway at the University. Applicants should preferably have substantial practical experience in recombinant DNA techniques, especially involving DNA sequencing and/or oligonucleotide synthesis. The appointees will have the opportunity to participate actively in specific ongoing research projects such as molecular virology, molecular endocrinology, protein engineering or plant genetics. Applicants should preferably have an Honour's Degree in an appropriate molecular biological science or a specific knowledge of synthetic nucleic acid chemistry. A postgraduate degree is not essential.

Salary range: £7,521 — £12,150 per annum with contributory pension rights under USS, initial placing depending on age and experience. Closing date: 10 August 1985. (Please quote Ref. 85/K.)

MOLECULAR BIOLOGIST

Department of Biochemistry

A graduate research assistant is required to investigate the molecular biology of mumps virus. The work will involve the molecular cloning of mumps virus genes and DNA base sequencing and will be part of the Molecular Virology Group led by Professor S J Martin. Candidates should preferably have a good honours degree in an appropriate biological science with a strong interest and motivation for research involving genetic engineering methods. This position is available for a three-year period from 1st October 1985, and is supported by a grant from the Medical Research Council. A suitable person would be encouraged to enrol for a further degree.

Salary range: £6,600 — £8,922 per annum, initial placing depending on age and experience.

Applications including curriculum vitae and the names and addresses of two referees should be sent to the Personnel Officer, at the address below. Closing date: 10th August, 1985. (Please quote ref. 85/P).

RESEARCH ASSISTANT

Department of Oncology

This post, funded by the Ulster Cancer Foundation, is tenable initially for two years commencing as soon as possible. The research involves screening human tumour samples with DNA viral probes. Candidates should preferably have a good honours degree and experience in recombinant DNA technology. A PhD degree would be advantageous although a suitable applicant may enrol for a higher degree.

Salary in the range: £7,521 — £8,922, initial placing depending on age and experience. (Please quote Ref. 85/M).

Applications including a full curriculum vitae and the names and addresses of two referees should be sent to the Personnel Officer, The Queen's University of Belfast, BT7 1NN, Northern Ireland. (7164)A

THE UNITED MEDICAL AND DENTAL SCHOOLS

GUY'S CAMPUS

LABORATORY SCIENTIFIC OFFICER

required in the

DEPARTMENT OF ANATOMY

to join a group working on regeneration within the Central Nervous System. The successful applicant will have considerable experience of neurohistological and immunocytochemical techniques, including all routine HRP developments.

The appointment is for three years. Salary on scale £6,726 — £7,159 per annum plus £1,084 per annum London Weighting.

Application Forms are available from the Staffing Officer, Guy's Hospital Medical School, London SE1 9RT, quoting Ref: G/A/82. (7169)A

UNIVERSITY OF GLASGOW

DEPARTMENT OF CHEMISTRY

POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for a Post-doctoral Research Assistantship, supported by the SERC for work on the Biosynthesis of quinolizidine alkaloids. Candidates should have a strong background in organic synthesis or natural product chemistry. The appointment will be available for two years, starting as soon as possible, at a salary within the range £7,520 — £8,450 per annum (range 1A under review, research staff scale) with USS benefits.

Applications, including a full curriculum vitae and the names of two referees should be sent to Dr D J Robins, Department of Chemistry, University of Glasgow, Glasgow G12 8QQ. (7161)A

DEPARTMENT OF ASTRONOMY University of Leicester

OBSERVATIONS OF PULSATING STARS

Applications are invited for a 3-year postdoctoral position tenable from October 1985. The research will involve optical and near infrared photometry of pulsating stars, particularly RR Lyrae variables, and measurement of their radial velocity curves. The aim being to determine radii and absolute magnitudes and thus a calibration of the distance scale. Previous experience of IR or optical photometry or optical spectroscopy would be an asset. Starting salary is in the range £7,520—£9,390, plus USS benefits, depending on age and experience.

Applicants should send a curriculum vitae with a list of publications and should arrange for two referees to write in their support. Applications and references should be received by 31 August and should be sent to: Dr. R.F. Jameson, Astronomy Department, The University, Leicester, LE1 7RH, to whom any further enquiries should also be directed.

Quasars and Active Galactic Nuclei

An SERC-supported postdoctoral research post will be available from October 1985 for work on the development of theoretical models of active galactic nuclei. The post is tenable for up to three years at a starting salary (under review) of up to £9,390 p.a., plus USS benefits, depending on qualifications and experience.

Applicants should send a curriculum vitae and list of publications, together with any other relevant information, and should arrange for two referees to write in their support. Applications and references should be received as soon as possible and not later than 31 August 1985. They should be addressed to: Dr. D.J. Raine, Astronomy Department, The University, Leicester, LE1 7RH, to whom any further enquiries should also be directed. (7134)A

THE UNIVERSITY OF SYDNEY CHAIR OF RESTORATIVE DENTISTRY Reference no 11/04

Applications are invited for the Chair of Restorative Dentistry which became vacant following the retirement of Professor J S Lyell, who held the Chair with the title of Operative Dentistry. The Chair is at present located in the Department of Operative Dentistry but the Faculty is currently considering a reconstruction of its departments and its clinical teaching.

The main teaching and research areas are operative dentistry, crown and bridgework, endodontics and dental materials science.

Applicants should have advanced clinical experience in one or more of these areas and hold a dental qualification which entitles them to practise dentistry in New South Wales.

Salary will be at the rate of \$A52,026 per annum and there is at present a clinical loading.

The University reserves the right not to proceed with any appointment for financial or other reasons.

Applications in duplicate, quoting reference no, including curriculum vitae, list of publications, and the names and addresses of three referees, to the Registrar, University of Sydney, NSW 2006, Australia, by 24 May 1985

Further information should be obtained from Secretary General, Association of Commonwealth Universities (Apts), 36 Gordon Square, London WC1H 0PF. (W2042)A

UNIVERSITY OF EDINBURGH

DEPARTMENT OF BACTERIOLOGY

(in collaboration with the Moredun Research Institute)

Applications are invited for a

POST-DOCTORAL RESEARCH ASSISTANT

to assist Dr I R Poxton and J A Spence (Moredun Research Institute) in a project funded by the Wellcome Trust entitled "A Study of Gram-negative Bacteria and Spirochaetes in Developing Broken Mouth of Sheep". This will involve the isolation and identification of anaerobic bacteria and spirochaetes from the mouths of diseased and healthy sheep, immunochemical characterisation of the bacteria and measurement of locally-produced and systemic antibodies.

Experience in anaerobic culture techniques is desirable and an interest in immunology or immunochemistry would be an advantage but is not essential. The position is for three years. Salary scale (Research Range 1A) £7,520—£8,920 (under review).

Further details from and applications (including the names of two academic referees), (four copies) to Dr Ian R Poxton, Bacteriology Department, University of Edinburgh Medical School, Teviot Place, Edinburgh, EH8 9AG Closing date for applications 31 August 1985.

Please quote reference 5264. (7159)A

THE UNIVERSITY OF MELBOURNE LECTURER (Continuing or Limited Tenure) in the

DEPARTMENT OF PHARMACOLOGY

Applications are invited for the above position. The successful applicant will be expected to have a PhD or equivalent qualification plus teaching and/or research experience in toxicology, but a well qualified pharmacologist who is prepared to embark on a primary commitment to toxicology could be considered. The appointee will be expected to carry out research, teach and otherwise practise as a toxicologist, but will also teach in other selected areas of pharmacology to medical, dental and science undergraduates

Further enquiries may be directed to Professor M J Rand in the University, telephone (03) 344 5673.

The commencement date for this position is in the period November 1985 to February 1986. Salary within the range \$A23,236—\$A34,467 per annum.

Further printed information regarding details of application procedure and conditions of appointment is available from the Appointments Officer (Academic), University of Melbourne, Parkville, Victoria 3052, Australia, or the Secretary General, Association of Commonwealth Universities (Apts), 36 Gordon Square, London WC1H 0PF.

Applications, in duplicate, including names and addresses of at least three referees and quoting position number 534 0020 should be addressed to the Staff Officer, University of Melbourne, and close on 30 September 1985.

Equal opportunity in employment is the policy of the University of Melbourne. Selection is based on merit and all applications will be assessed regardless of sex, race, ethnicity or disability.

Applications from both men and women are invited for all vacancies. (W2043)A

UNIVERSITY OF CAMBRIDGE Department of Physics POSTDOCTORAL RESEARCH ASSISTANTS

Applications are invited for two posts for work on polymer and colloid physics. The project will focus on interactions between polymers and surfactants at interfaces which will be studied using direct force measurement techniques and other complementary methods. Applicants should have a PhD in Physics or Chemistry and preferably experience in colloid/interface science. The posts are tenable up to two years. Salary within the range £7,520—£9,860 pa.

Applications including cv and names of two referees should be sent to The Secretary, Cavendish Laboratory, Madingley Road, Cambridge CB3 0HE. (7165)A

MONASH UNIVERSITY CENTRE FOR EARLY HUMAN DEVELOPMENT RESEARCH ENDOCRINOLOGIST Monash-IVF Programme

Duties include: (1) Supervision of routine IVF hormone assays, (2) Development of experimental techniques to expand the Endocrine research component of this IVF programme and (3) Liaison with clinical colleagues and motivation of junior staff within the IVF programme. Appointee would have basic endocrine research facilities and possible appointment as Research Fellow in the Centre for Early Human Development and Department of Obstetrics and Gynaecology. Applicants must have a PhD and good research record in basic and laboratory endocrinology. Salary: \$A22,614—\$A34,467 pa

Enquiries to Dr A Trouson, Centre for Early Human Development, Queen Victoria Medical Centre, 172 Lonsdale Street Melbourne, Australia 3000.

Applications including Ref no 32962, curriculum vitae and referees to the Registrar, Monash University, Clayton, Vic 3168 Australia by 31/8/85

An equal opportunity employer. (W2045)A

THE ANIMAL VIRUS RESEARCH INSTITUTE SCIENTIFIC OFFICER

Applications are invited for the post of Scientific Officer to work in a group investigating the molecular biology of African Swine Fever virus. This virus contains a complex double-stranded DNA genome. The project involves analysis of the structure and function of the viral genome, using recombinant DNA methods and associated techniques. Collaboration with other groups within the Institute studying the immunology and pathogenicity of the virus is involved. The successful candidate will have a First or upper second class honours degree in a biological science, experience in molecular biology techniques is desirable.

Salary scale. £6,190—£8,561.

22 days annual holiday, non-contributory superannuation and sickness benefit schemes. A house may be available for a suitable, married applicant.

Applications to include curriculum vitae and the names of two referees to be sent as soon as possible to the Secretary, The Animal Virus Research Institute, Ash Road Pirbright, Woking, Surrey GU24 0NF. Tel: 0483 232441

The Animal Virus Research Institute is an Equal Opportunity Employer. (7156)A

Please mention
nature
when replying to
these advertisements

THE UNIVERSITY OF LEEDS

DEPARTMENT OF PHYSIOLOGY POSTDOCTORAL RESEARCH FELLOW

Applications are invited for the above post for work on the neural control of cilia in *Lymnaea* involving the use of electron-microscopy and electrophysiological techniques.

A good honours degree in Physiology, Zoology or Anatomy and a PhD or equivalent experience in a relevant subject are required. Candidates should also have postgraduate experience of ultrastructural techniques.

Post available from 1 October 1985 for a fixed period of up to three years.

Salary within the range £7,520 — £8,450 (under review) on the 1A range for Research and Analogous staff, according to age, qualification and experience.

Informal enquiries may be made to Dr W Winlow (tel (0532) 431751 ext 7443 or 7165).

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT quoting reference number 94/14. Closing date for applications 16 August 1985. (7167)A

NUTRITIONIST

The Medical Research Council

wishes to appoint a Nutritionist to take part in the range of dietary studies undertaken by the Environmental Epidemiology Unit. The Unit uses epidemiological methods to investigate the influence of the environment in the aetiology of chronic disease. The main programmes of research are dietary and industrial. The Unit is associated with the Faculty of Medicine, Southampton University and is sited at Southampton General Hospital.

The appointment will be for three years in the first instance, to the MRC scientific staff on a salary scale comparable to University academic salaries; and at a point appropriate to age and experience.

Further information and application details may be obtained from the Director, MRC Environmental Epidemiology Unit, Southampton General Hospital, Southampton SO9 4XY

Closing date for applications August 30th. (7114)A

MEDICAL RESEARCH COUNCIL

Laboratory of Molecular Biology, Cambridge

ORGANIC CHEMIST

Applications are invited from post-doctoral scientists with a strongly developed interest in bioorganic chemistry to join a group working in the peptide and oligonucleotide field. Previous experience in peptide synthesis would be an advantage but is not essential.

L'UNIVERSITE DE LAUSANNE

ouvre une inscription en vue de pourvoir, dès le 1er septembre 1986, un poste de

PROFESSOR ASSOCIE

rattaché à l'Institut de Zoologie et d'Ecologie animale (IZEA) de la Faculté des sciences.

Il s'agit d'un poste à plein temps, comportant de l'enseignement (écologie quantitative, génétique des populations et spéciations) et de la recherche (relations phylétiques et écologie des mammifères).

Les candidatures (curriculum vitae, liste des publications, tirés à part des publications les plus significatives) doivent parvenir avant le 30 septembre 1985 au professeur Bernard TESTA, doyen de la Faculté des sciences, Collège préposé, 1015 Lausanne-Suisse. (W1993)A

SOUTH MANCHESTER HEALTH AUTHORITY

Christie Hospital and Holt Radium Institute Paterson Laboratories POST-DOCTORAL RESEARCH FELLOW

A post-doctoral position is available in the Department of Radiation Biology for a cell biologist with an interest in endothelium and with experience in culture techniques required for the investigation of long-term cytotoxic injury in bone marrow

Initial salary will be in the range £8,450 to £11,205 per annum (under review), depending upon age and experience and the initial term of the appointment will be 3 years.

Applications, together with cv, and the names of 2 academic referees, should be forwarded to Dr J H Hendry, Department of Radiation Biology, Paterson Laboratories, Christie Hospital & Holt Radium Institute, Wilmslow Road, Manchester M20 9BX. (7162)A

The post is available for 3 years from September 1st or as soon as possible thereafter. Salary in the range £8,920 to £14,925 pa. Further information from Dr R C Sheppard (Cambridge) (0223) 248011).

Applications — including a full CV, a short statement on relevant experience and the names of two professional referees — should be sent by (August 8th 1985), quoting reference RS/85 to: The Assistant Director, MRC Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH. (7166)A

UNIVERSITY OF BRISTOL LONG ASHTON RESEARCH STATION HEAD OF WEED RESEARCH DIVISION

An experienced scientist is required to lead and to manage the Weed Research Division, which is to be established at Long Ashton Research Station. The Division will have national responsibility for fundamental, strategic and applied research on weeds and weed control and associated areas of vegetation management. The Division will need to collaborate extensively with others both internally and externally.

While the minimum qualification is a good Honours Degree or equivalent, no applicant is likely to be appointed who has not been awarded a Higher Degree and gained a well established reputation in relevant scientific disciplines. The post requires an understanding of the administration of research and the experience and personality to ensure the respect of expert staff and to provide them leadership.

Appointment is in Grade 6 (£17,000 to £22,926 per annum) Non-contributory superannuation scheme.

Further particulars from: The Secretary, Long Ashton Research Station, Long Ashton, Bristol, BS18 9AF. Written applications, with a CV and the names of three referees must be received by Thursday, 1st August, 1985. (7145)A

POST-DOCTORAL POSITION

available after 1 October

Cell biology-physiology-biochemistry laboratory studying isolated cardiac membrane channel proteins seeks

IMMUNOLOGIST OR PROTEIN BIOCHEMIST

with immunological experience for immunofluorescence and immunoelectron microscopic investigations. Applicant should have doctorate and experience with poly- and monoclonal antibody techniques.

Send curriculum vitae, description of research interests, references to: Dr. Ernest Page, Department of Medicine, BH Box 137, The University of Chicago, 5841 South Maryland Avenue, Chicago, Illinois 60637. Equal Opportunity/Affirmative Action Employer. (NW45)A

UNIVERSITY OF WALES COLLEGE OF MEDICINE DEPARTMENT OF MEDICINE PhD SCHOLARSHIP

available in the above Department as soon as possible to work in the general field of neuroendocrinology/neurobiology. The areas include the application of radioimmunoassay and HPLC and neurone culture to the study of hypothalamic and other brain neuropeptides. Work also includes the study of the applications of neurotransmitters and neuro-modulators to the control of endocrine function in general and neuroendocrine function in particular. Other methodology involves the measurement of catecholamines by HPLC with electrochemical detection.

Applications, in the form of a curriculum vitae with the names and addresses of two referees and quoting Ref No M6/99 should be submitted to the Registrar and Secretary, Personnel Office, University of Wales College of Medicine, Heath Park, Cardiff, CF4 4XN by 9th August, 1985.

Further information available from Dr Maurice Scanlon (Tel No 0222/755944 Ext 2182) (7148)A

UNIVERSITY OF OXFORD DEPARTMENT OF BIOCHEMISTRY Genetics Laboratory POSTDOCTORAL RESEARCH ASSOCIATE — GRADE 1A (Ref: G/R/132)

A postdoctoral research assistant is needed to organise a DNA bank, to assist in an associated cell bank, and to undertake restriction enzyme and allied analyses of families relevant to Duchenne's muscular dystrophy. All these techniques are well established in the laboratory. The position, funded by the Muscular Dystrophy Group, will be for one year in the first instance, but is expected to continue for at least a further year. Graduates with relevant experience will be considered. Exceptional candidates would be considered for part-time work.

Salary on the scale (or pro rata to) £7,520 — £9,860 pa (under review) depending on age and experience.

Applications, including the names and addresses of two referees should be sent, as soon as possible, to: The Administrator, Department of Biochemistry, South Parks Road, Oxford. OX1 3QU (7124)A

**PUBLIC HEALTH
LABORATORY
SERVICE BOARD**

Phls Centre for
Applied Microbiology
& Research

Vaccine Research & Production
Laboratory

RESEARCH ASSISTANT

Required in the Vaccine Research and Production Laboratory at the Centre for Applied Microbiology and Research, Porton Down. Duties involve the characterization of monoclonal antibodies for potential use in the diagnosis and study of the antigenic structure of human cytomegalovirus, to be carried out in association with the Department of Medical Microbiology, St Georges Hospital, London. The appointment is to run for one year with effect from October 1985.

Applicants should have obtained, or expect to obtain, an honours degree in an appropriate subject, must be under the age of 32 and have studies or worked continuously for the past three years in a university or hospital in the United Kingdom

Salary in the range £6,559-£8,725

National Health Service terms and conditions apply

Application forms from the Personnel Officer, PHLS Centre for Applied Microbiology & Research, Porton Down, Salisbury, Wilts SP4 0JG to be returned by 15th August 1985.

Please quote Reference No 82 when applying. (7142)A

**THE UNIVERSITY OF
LEEDS**

DEPARTMENT OF BIOCHEMISTRY

**PREDOCTORAL
RESEARCH ASSISTANT**

Applications are invited as soon as possible for the above post in the MRC Membrane Peptidase Research Group for work on studies of a panel of monoclonal antibodies recognising antigens in renal and intestinal brush border membranes as well as in generating new hybridomas. The techniques include those of mammalian cell culture, immunocytochemistry and those involved in characterizing the antigens recognised. Candidates should have a degree in an appropriate subject and some relevant laboratory experience.

The post is available from 1 September 1985 for a fixed period of up to thirteen months

Salary on the IB Range for Research and Analogous Staff (£6,600 — £8,920 (under review), according to age, qualifications and experience.

Informal enquiries may be made to Dr A J Kenny (tel (0532) 431751 ext 7438).

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference no 83/63. Closing date for applications 1 August 1985. (7153)A

LA TROBE UNIVERSITY
Melbourne, Australia

**School of Physical Sciences
LECTURER IN GEOLOGY
(GEOLOGIST/
GEOPHYSICIST)
(TENURABLE)**

Applications are sought for a tenurable lectureship in the Department of Geology. Applicants should have a PhD or equivalent applied experience in either geology or geophysics and expertise in the application of geophysics to geological problems. Possible areas of research interest could include petroleum or mineral exploration, seismic stratigraphy, geology and geophysics of the ocean basins or structure and geophysics of the continental crust. The successful applicant will be expected to establish a geophysics program for senior undergraduates and to carry out research and supervision of post-graduate students in the field of interest.

Salary: \$A26,236 — \$A34,467.

Applications, marked 'Confidential' and quoting reference number A0/024/013, close with the Staff Officer, La Trobe University, Bundoora, Victoria, Australia 3083, on 16 August 1985. Candidates in UK or Europe should also send a copy of their application to the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, from whom further information is available (W2034)A

**CONFERENCES &
COURSES**

**UNIVERSITY OF READING
DEPARTMENT OF SOIL SCIENCE
MSc COURSE IN
SOIL SCIENCE: SOIL
WATER MANAGEMENT**

Places are available on this new course starting in October 1985. It lasts one or two years depending on whether project work is undertaken in Reading or overseas.

The purpose of the course is to provide training in the efficient management of water in soils, especially in arid and semi-arid regions. Specialized courses are provided in soil physics, the behaviour of water in soils, agricultural meteorology, water relations of crops, and the management of soil water for crop production in rain-fed agriculture. During the course, emphasis will be placed on instrumentation and measurement techniques leading to the effective management of water. Project work can be undertaken overseas where appropriate arrangements can be made.

Applications are invited from graduates with a science background. No research council studentships can be offered for entry in October 1985.

Enquiries to the Secretary, Department of Soil Science, University of Reading, London Road, Reading RG1 5AQ. (7173)C

**THE UNIVERSITY OF
LEEDS**

**Biotechnology Unit
h9110MEMBRANE-PROTEIN
ENGINEERING**

**Applications are invited for a post of
POSTDOCTORAL
RESEARCH FELLOW**

to work on a protein engineering project to study membrane anchor regions in hybrid enzymes. This work will involve recombinant DNA methods (including the use of oligonucleotides), enzymological and immunological techniques.

Applicants should have a PhD in Biochemistry, Genetics or a related field preferably with experience in membrane protein biochemistry and experience in, or willingness to learn recombinant DNA techniques.

Post available from 1 October 1985 for a fixed period of up to two years.

Salary within the range £7,520 — £9,860 (under review) on the 1A Range for Research and Analogous Staff according to age, qualifications and experience.

Informal enquiries may be made to Dr M J McPherson (tel (0532) 431751 ext 402).

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference number 201/5. Closing date for applications 8 August 1985. (7122)A

FELLOWSHIPS

**UNIVERSITY OF
EDINBURGH**

**DEPARTMENT OF BIOCHEMISTRY
SERC POSTDOCTORAL
FELLOWSHIP**

Applications are invited for a SERC supported postdoctoral fellowship to work on refinement of the molecular structure of collagen and analysis of interactions of collagen with other molecules. Experimental work will involve use of synchrotron radiation, molecular graphics and computing. New specimen preparation procedures are being developed and a low-temperature device constructed. The department is well equipped for the relevant experimental work.

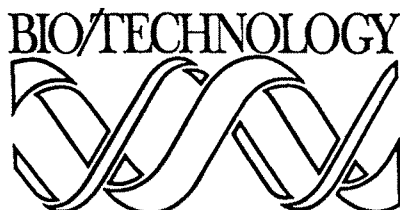
The position is for three years starting salary on range £7,520 — £8,920 (under review), and is available immediately.

Please apply to Professor J. Miller, Department of Biochemistry, University of Edinburgh Medical School, Hugh Robson Building, George Square, Edinburgh, EH8 9XD, enclosing curriculum vitae and the names and addresses of two referees.

Please quote reference 5265 (7157)E

ATTENTION: R&D MANAGERS, PRINCIPAL
INVESTIGATORS, DEPARTMENT CHAIRPEOPLE,
SENIOR SCIENTISTS

**BIO/TECHNOLOGY announces its
premier 3-day conference**



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Save \$75 by registering early

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- Day 3: Innovations in Pharmaceuticals & Immunology

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FELLOWSHIPS

UNIVERSITY OF NOTTINGHAM MEDICAL SCHOOL

Department of Biochemistry POST-DOCTORAL FELLOW

Applications are invited for a research fellowship financed by the British Heart Foundation for a study of phosphoinositide metabolism and the molecular mechanisms mediating the responses of cardiac muscarinic receptors. The fellowship is available for two years initially from 1 October, 1985 at a starting salary of £7,980 or £8,450 depending on age and experience. Membership of USS included.

Applicants should send curriculum vitae and the names of two referees to Professor J N Hawthorne, Department of Biochemistry, Medical School, Queen's Medical Centre, Nottingham NG7 2UH, from whom further details may be obtained (telephone: 0602-700111 ext 3488). Closing date 31st August, 1985. (7115)E

COLEG PRIFYSGOL GOGLEDD CYMRU University College of North Wales, Bangor

DEPARTMENT OF BIOCHEMISTRY AND SOIL SCIENCE POSTDOCTORAL FELLOWSHIP

Salary:— £7,520 — £9,390 per annum

Applications are invited for the above position to study the control of sugar accumulation in sugar beet storage tissue. The work will involve use of the micropressure probe to measure the pressure dependence of ion fluxes as well as more conventional biochemical and physiological techniques.

The post is funded by the AFRC starting on the 1st October, 1985, until end of February 1987, and the successful applicant should have a PhD with a strong background in plant physiology or biochemistry and an interest in biophysical methods.

Applications (two copies), giving details of age, education and experience together with the names and addresses of two referees should be sent to the Personnel Office, University College of North Wales, Bangor, Gwynedd LL57 2DG, from whom further particulars may be obtained. Informal enquiries may be made to either Professor R G Wyn Jones or Dr A D Tomos, Department of Biochemistry and Soil Science. Telephone Bangor (0248) 351151, Extension 359/304.

Closing date for applications: 1st August, 1985. (7117)E

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IMPERIAL CANCER RESEARCH FUND RESEARCH FELLOWSHIP

A post-doctoral, three year appointment is available in our Cellular Pharmacology and Antitumour Chemistry Laboratory, to work with Dr A Creighton on projects involving tumour therapy with antibody targeted radionuclides.

Salary range £10,285 to £12,570, entry according to qualifications and experience. Further details are available from Ms S M Hurley, Imperial Cancer Research Fund, Lincoln's Inn Fields, London, WC2A 3PX, telephone 01 242 0200 ext 305 quoting reference: 93/85. (7147)E

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE (University of London)

DEPARTMENT OF BIOCHEMISTRY AND CHEMISTRY JUNIOR RESEARCH FELLOWSHIP

Applications are invited from graduates on students obtaining a good honours degree in Biochemistry or related subjects for a three year Junior Research Fellowship, starting on 1 October 1985. The successful candidate will register for a higher degree in the University of London. The project concerns the mechanisms of polyphosphoinositide metabolism and arachidonic acid release in endocrine cells. The conditions of employment and salary are similar to the SERC/MRC studentships.

A curriculum vitae and the names of two academic referees should be sent to Professor B.A. Cooke, Department of Biochemistry and Chemistry, Royal Free Hospital School of Medicine, Rowland Hill Street, London, NW3 2PF. Telephone 01-794-0500 extension 4202.

(7116)E

UNIVERSITY COLLEGE DUBLIN

Departments of Zoology and Botany

POSTDOCTORAL FELLOWSHIP

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Send curriculum vitae with the names of two referees to: Dr Michael F Ryan, Department of Zoology, University College Dublin, Belfield, Dublin, 4, by 10th August, 1985. (7174)E

EMBO European Molecular Biology Organization LONG TERM FELLOWSHIPS IN MOLECULAR BIOLOGY Autumn 1985 — Awards

Next deadline: 15 August 1985

EMBO long term post-doctoral fellowships are awarded to promote the development of molecular biology and allied research in Europe and Israel. To be eligible a candidate must hold a doctorate degree and the exchange must involve a laboratory in Western Europe or Israel. EMBO fellowships are not, however, awarded for exchanges between laboratories within any one country. Long term fellowships are awarded initially for one year, but depending on a review of progress, they may be renewed for a second year. In cases of exceptional scientific merit renewal for a third year is possible. The fellowship comprises a return travel allowance for the fellow and any dependents and a stipend and dependents' allowance.

Since the selection procedure may include an interview, candidates are requested to respect the deadline for complete applications which is 15 August 1985. Successful candidates will be notified of their awards immediately after the meeting of the selection committee, which is on 21 October 1985.

Application forms and further details may be obtained from Dr. J. Tooze, Executive Secretary, European Molecular Biology Organization, Postfach 1022.40, 69 Heidelberg 1, F. R. Germany. (W1893)E

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The European Southern Observatory (ESO) intends to award up to six fellowships tenable in the ESO Scientific-Technical Center which is located in Garching near Munich.

The main areas of activity are:

- to carry out a program of development of auxiliary instrumentation for the La Silla telescopes;
- to develop plans for future telescopes involving new technology;
- to make studies in observational and theoretical astrophysics so that the observing facilities may be used in an optimal way;
- to provide data reduction facilities to users of the ESO instruments;
- to provide photographic facilities for atlases of the southern sky;
- to foster cooperation in astronomy and astrophysics in Europe.

In addition there is the possibility of participating in the activities of the European Coordinating Facility of the Space Telescope (ST-ECF) which has recently been established at ESO.

Most of the scientists in the Center come from the Member States of ESO, but several are from other countries. The Member States of ESO are: Belgium, Denmark, the Federal Republic of Germany, France, Italy, the Netherlands, Sweden, and Switzerland. In addition to regular staff members, the Center comprises visiting scientists and postdoctoral fellows.

ESO facilities include the La Silla Observatory in Chile with its seven telescopes in the range of 0.9 to 3.6m, as well as a 1m Schmidt and some smaller instruments. In Garching, extensive measuring, image processing and computing facilities are available.

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Enquiries, requests for application forms and applications should be addressed to:



European Southern Observatory
Fellowship Program
Karl-Schwarzschild-Strasse 2
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(W2041)E

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Applications are invited from final year students expecting to graduate with a first or upper second class honours degree in appropriate branches of science, medicine or veterinary medicine. The successful candidate will work on one of the following topics: the role of opioids in the control of neuropeptide release into hypophyseal portal blood; identification of neuropeptide receptors on living neurones by video-intensification microscopy (VIM); molecular biology of the LHRH receptor; biogenesis of the tachykinins in the nervous system. The candidate will be registered as a research student in the University of Edinburgh.

Applications including a full curriculum vitae together with the names of 3 referees should be addressed to the Director, Professor G. Fink, MRC Brain Metabolism Unit, University Department of Pharmacology, 1 George Square, Edinburgh EH8 9JZ as soon as possible. (7131)F

**MRC BRAIN METABOLISM UNIT,
University Department of Pharmacology,
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RESEARCH STUDENTSHIP**

Applications are invited from final year students expecting to graduate with a first or upper second class honours degree in appropriate branches of science, medicine or veterinary medicine. The successful candidate will work on one of the following topics: the role of opioids in the control of neuropeptide release into hypophyseal portal blood; identification of neuropeptide receptors on living neurones by video-intensification microscopy (VIM); molecular biology of the LHRH receptor; biogenesis of the tachykinins in the nervous system. The candidate will be registered as a research student in the University of Edinburgh.

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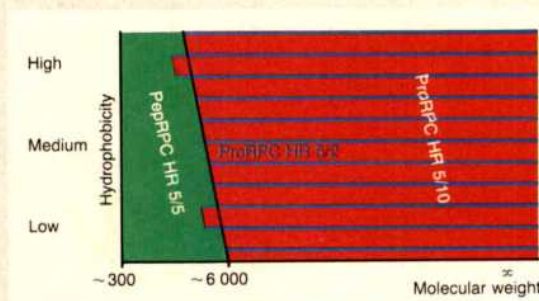


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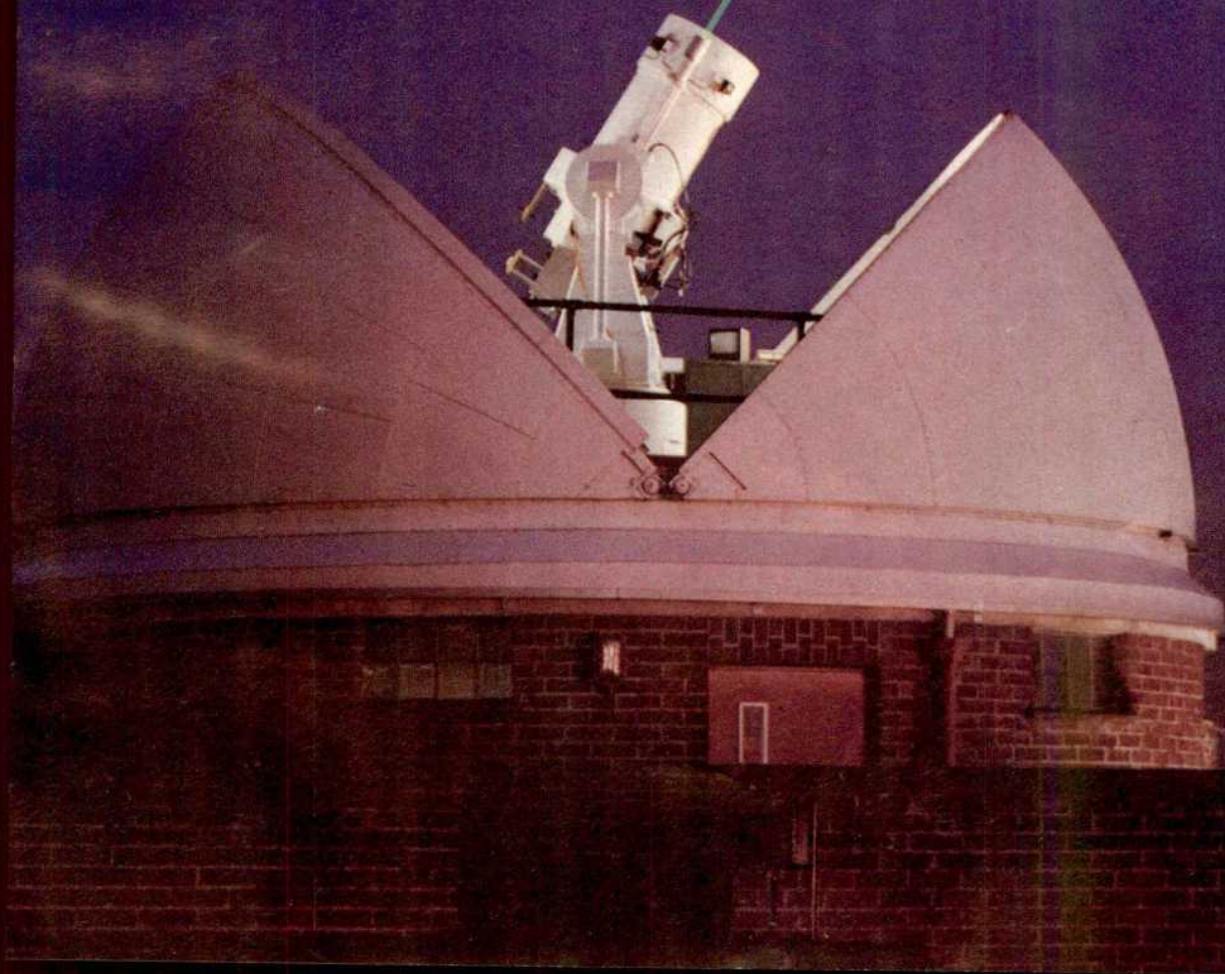
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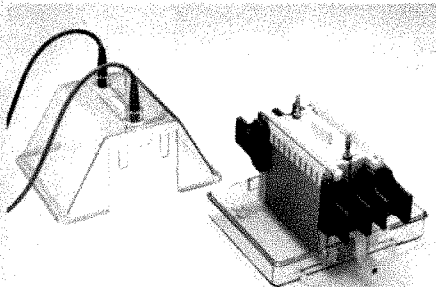
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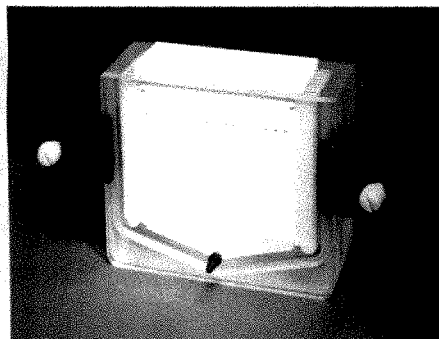
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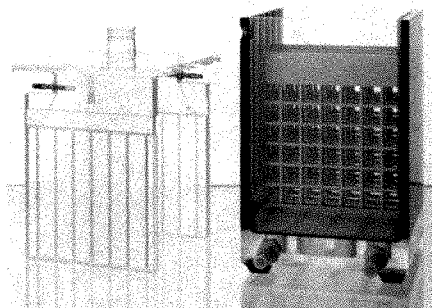
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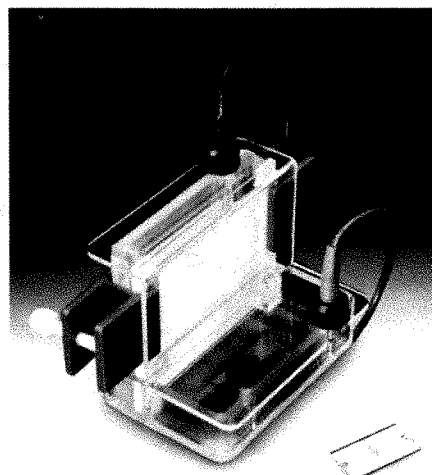
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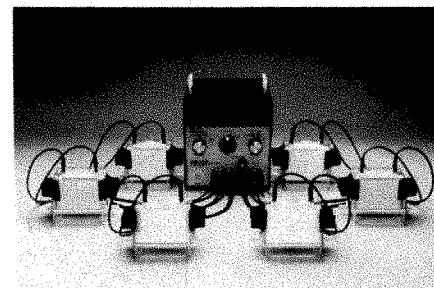
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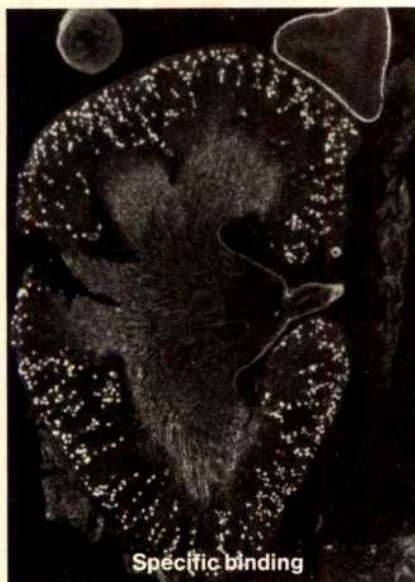
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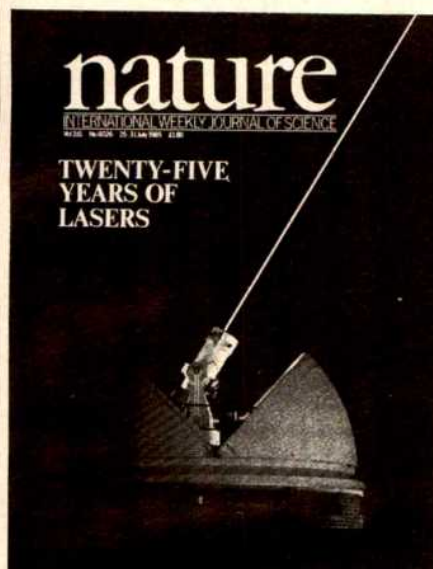
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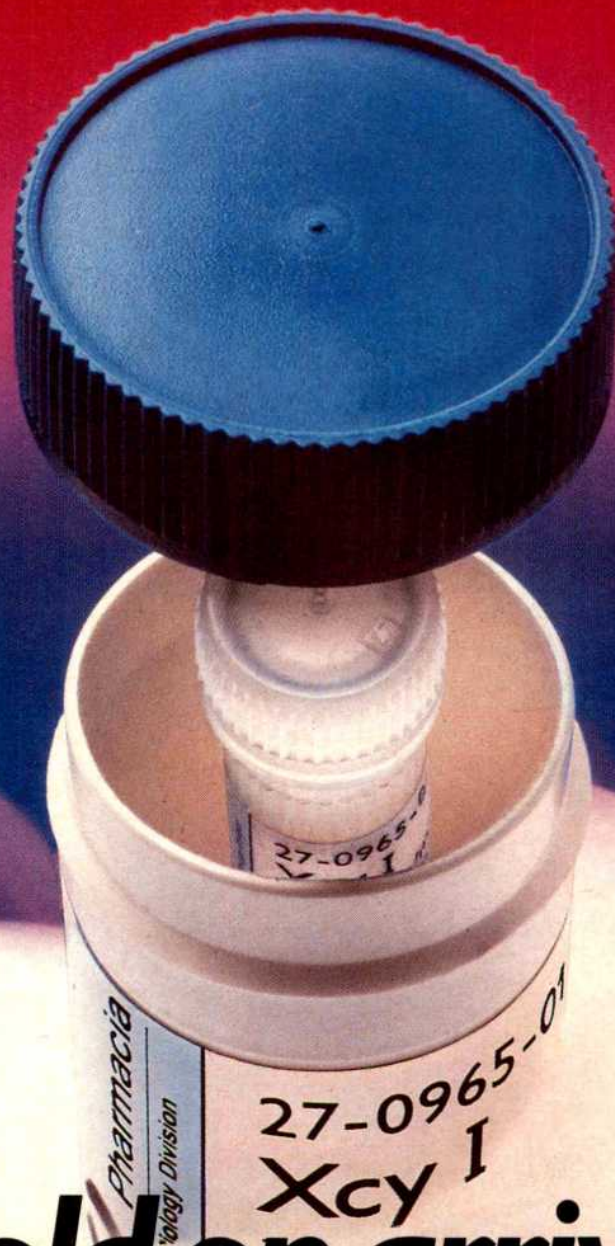
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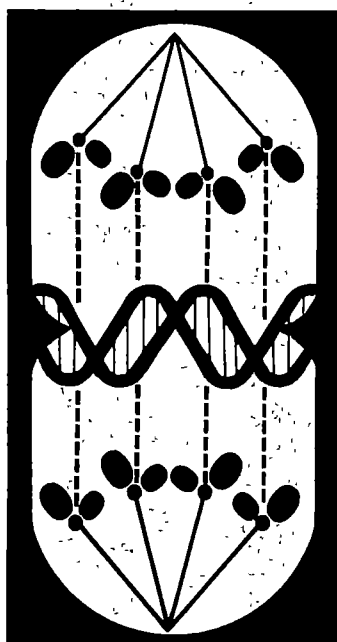
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Genes and Systems in Development

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8 45-8 50	Chairman	
8 50-9 40	<i>Drosophila</i> early development	C. Nüsslein-Volhard, Max Planck, Tübingen
9 40-10 30	Genetic control of <i>Drosophila</i> development	D. Hogness, Stanford University
10 30-11 10	COFFEE	
11 10-12 00	Comparative expression of homeobox genes in fly and mouse	W. McGinnis, Yale University
12 00-1 30	LUNCH	
1 30	Chairman	
1 30-2 15	Cytoplasmic localizations in the amphibian egg	J. Gerhart, University of California, Berkeley
2 15-3 00	Genes that control nematode cell lineage	H.R. Horvitz, Massachusetts Institute of Technology
3 00-4 00	ROUND TABLE with day's speakers	
4 00-4 30	TEA	
4 30-5 30	Closing talk	G. Stent, University of California, Berkeley

DAY 2 TUESDAY, 8 OCTOBER

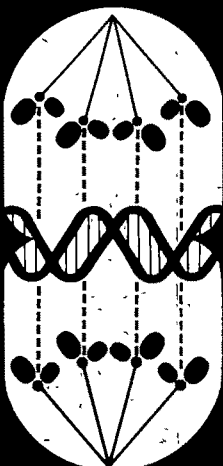
8 45-8 50	Chairman	
8 50-9 40	A master regulatory locus that determines cell specialization in yeast	I. Herskowitz, University of California, San Francisco
9 40-10 30	Regulatory proteins in bacteria and yeast	M. Ptashne, Harvard University
10 30-11 10	COFFEE	

11 10-12 00	Simple and combinatorial regulation of gene transcription	K. Yamamoto, University of California, San Francisco
12 00-1 30	LUNCH	
1 30	Chairman	
1 30-2 15	Control of animal growth by gene transfer	R. Palmiter, University of Washington, Seattle
2 15-3 00	Models for human gene therapy	T. Friedmann, University of California, San Diego
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9 40-10 30	Expression of immunoglobulin genes in transgenic mice	U. Storb, University of Washington, Seattle
10 30-11 10	COFFEE	
11 10-12 00	Proto-oncogenes: the good and the bad	J.M. Bishop, University of California, San Francisco
12 00-1 30	LUNCH	
1 30	Chairman	
1 30-2 15	Cell recognition during neuronal development	C. Goodman, Stanford University
2 15-3 00	Molecular insights into mammalian systems	F. Bloom, Research Institute of Scripps Clinic
3 00-4 00	ROUND TABLE with day's speakers	
4 00-4 30	TEA	
4 30-5 30	Closing talk	D.W. Martin, Genentech

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Where next after Geneva break?

The Geneva arms control talks have adjourned without breaking down, which is something to be grateful for. The negotiators should spend their break deciding where to go from here.

THE prospect that something will come out of the bilateral arms control negotiations at Geneva, far from being dimmed by last week's adjournment, is enhanced because they have not long since broken down. That the negotiations would be difficult has been clear from the start even though those that broke down at the end of 1983 covered some of the same ground. This time, the problems at Geneva have been complicated by its announcement of the US plan to build a defence against strategic missiles, an issue that seems not unduly to have alarmed the Soviet Union during the earlier abortive talks. The best hope now is that the two sides will use the summer to work out the skeleton of an agreement that might be put to Mr Reagan and Mr Gorbachev when they meet, as they intend next November. But even that is a lot to hope for.

The present position is both complicated and delicate. The United States, which spent the years preceding the Anti-Ballistic Missile (ABM) Treaty of 1972 in trying (successfully, in the end) to convince the Soviet Union that ballistic missile defences would be dangerous, is now faced with the task of demonstrating that the opposite is the truth, that a workable shield against hostile missiles would be a boon. Inevitably, the logic must sound thin. Yet the United States is within its rights to insist that research programmes cannot be the subject of international treaties (because verification is impossible), while the Soviet Union can rightly say that some of the testing planned by the Strategic Defense Initiative (SDI) would violate the ABM treaty, as would the emplacement in an Earth orbit of a radar system for detecting hostile missiles, whether or not it was accompanied by a device for shooting down unwanted objects.

Understanding

The signs are that the Soviet Union is now willing to accept that research cannot be prevented by bilateral agreements. What the United States should in return be willing to accept is that successful research cannot be followed by development without violating the 1972 treaty so the need is for some general understanding (not at this stage a treaty) on mechanisms for modifying the ABM treaty by agreement. That, in any case, is about the only way in which the United States could hope to win support in Western Europe for its plans, and also the best way to avoid the increasingly bruising squabbles in Congress about money for SDI.

But how is it possible to agree in advance on procedures for modifying a treaty without knowing what changes will be required? The difficulties are fewer than they appear. The ABM treaty itself is, by its symmetry, a useful guide. In the original version, each side was allowed to protect only two regional targets, one city and one missile field. Each was allowed to build phased array radars, but only on its periphery, and directed outwards. There were restrictions (whose observance is not

easily verified) on the uses that might be made of anti-aircraft missiles. The general principle was that each side was allowed to follow, if it chose, prescribed courses of action. (In the event, the United States decided not to take up the right to deploy anti-missile missiles, the Soviet Union exercised only one of its two options.) So it might sensibly be agreed that any later modifications of the ABM treaty would follow the same principles. The first practical component to emerge from SDI is almost certain to be a recipe for an orbiting early-warning system which, if not accompanied by defences, could do no harm. So why not agree now to agree later on how such a system should be specified, and agree that each side should be entitled to one of them? And so on with the other planned components of SDI, as and if they become practical realities?

The rest of the Geneva agenda similarly cries out for re-examination. The serious flaw in the abortive negotiations of 1983 was that strategic and intermediate-range missiles were rigidly kept separate. The separation is now less complete, which is just as well when it is reckoned that missiles such as the Soviet SS20 and heavy bombers based in Europe have a similar strategic function in a European context. One obvious difficulty now is that so much time has passed since the Salt II agreement (1978) that each side probably needs, for good military reasons, a more modern strategic missile. The US need of something more credible than the MX missile stands out a mile. So here again is an opportunity for reciprocal modification of the existing agreements — permitted modernization coupled, say, with a target number for total warheads reducing steadily over some specific period ahead, something like a decade. There would be opportunities in such an arrangement to use the "build-down" principle in fashion two years ago — one new missile for each two old missiles destroyed. An arrangement like this would let ordinary people sleep more easily in their beds, but also keep the generals happy. But why bother about them? Because an agreement that lets one side feel permanently at a disadvantage will not stick. □

Reaganomics found out

The US economy is heading for stagnation and the need to cut the deficit is more urgent.

BUDGET directors, wherever they work, are not often popular, so that there are rarely floods of tears when they quit their jobs. The departure last week of Mr David Stockman, director of the US Office of Management and Budget since 1981, should be differently regarded. His continued presence at the White House has been for the past three years the best chance that something would be done to reduce the federal government's budget deficit, expected to exceed \$200 million in the year beginning on 1 October. The chance, recently only an off-chance, has no doubt been further diminished by President Ronald Reagan's abdominal operation, while the past week has seen such a confusion of signals about the condition of the US economy from the stock markets and the foreign exchanges that the most resolute members of the US Congress are likely to be given pause. However, this is not the time for masterly inaction.

Biological manuscripts

Contributors are reminded that, with the transfer of the Biological Sciences Editor to the Washington office, it will be helpful if they will in future send **four copies of all manuscripts** offered for publication, either (as at present) to London or Washington.

The past five years have been a topsy-turvy period when it seems to have been possible to make water run uphill. At the outset of the administration, the objective was to cut taxes, in the belief that an industrious industrial economy would make such good use of the extra money that wages and salaries would rise several times as much, thus restoring the federal government's revenues. Taxes were indeed cut, and there has been a remarkable increase in the number of potential taxpayers at work in the United States. But tax incomes have not increased to bridge the revenue gap. So the second and unplanned phase of Reaganomics, most of the past three years, has been a period during which the federal government has bridged the gap of borrowing in the markets, ultimately from overseas.

Inflation has been restrained by the determination to borrow (rather than to print money), economic growth has picked up in spite of historically high interest rates (necessary to tempt in funds from overseas) and the US dollar has been over-valued. The trade deficit, likely to exceed \$140,000 million during this calendar year, is the other side of the same coin, but that is the reason why the pace of economic growth has now declined almost to nothing, at least for the first half of this year. And now, not surprisingly, the value of the dollar has begun to fall. The third phase of Reaganomics has begun. We can only wait and see what it will be like.

The immediate temptation in Washington will be to go slow on attempts to cut the budget deficit. Members of the Congress who have been sharpening their knives will now be given pause by the apparent lack of growth between the first half of 1984 and the first half of this. The Keynesians among them and many others will be saying that a time of stagnation is the wrong time to be taking money out of the system. The hitch in that argument is that if the deficit persists, it will be necessary to increase interest rates again, which may have the same effect, putting a drag on US industry and intensifying the stagnation now in prospect. This is what Mr Paul Volcker, the chairman of the Federal Reserve System, was saying in Washington last week. The alternative would be to embrace inflation, which could easily become the hallmark of phase three.

That is why the United States has no choice but to look overseas for a solution. Japan and, latterly, West Germany have been earning large surpluses in their overseas trade, chiefly from that with the United States, and have obligingly lent that back to the United States. In principle, both governments (and some others) could help avoid the present prospect by allowing their own domestic demand to increase, either by lowering interest rates or (better not) by apeing the first phase of Reaganomics. But none of this is arithmetically possible unless the US budget deficit is cut. How else could Japan and West Germany earn the surpluses with which the US Treasury expects them to bridge its gap? The simple moral is that there should be a negotiation between the three countries principally concerned to decide which deficits should be cut by how much. The pity is that the chance to do this, last month's meeting in Bonn, has been let slip. And next year's meeting in Tokyo is too far away. □

University on brink

The changed University of London could be fun to watch.

The University of London is, or at least was, a remarkable institution. It is the only really large university in Britain, with more than 20,000 undergraduates. Not so long ago, it had no fewer than a dozen distinct medical schools, and a similar number of medically oriented postgraduate institutions. There is also a handful of specialized centres within the university concerned with matters as different as oriental languages and fine art. Until a quarter of a century ago, the University of London played a kind of authenticating role in British university life, acting as the validating umbrella beneath which sheltered a number of institutions that are now universities in their own right. During the same period, the university also ran single-handed a system of

public examinations for secondary schools throughout what was then the British Commonwealth, a function that has been eroded as much by the wish of Commonwealth countries to run their own affairs as by the university's choice.

The past few years have seen even more rapid change, largely because of the shortage of funds. Five years ago, a committee under Sir Brian (now Lord) Flowers advocated that the number of medical schools should be reduced, by mergers, to a half, and there have indeed been some moves in that direction. More recently, the main teaching establishments of the university have also been reduced in number. Small Bedford College has moved out into the country to merge with another small teaching school, Royal Holloway College, on a site more than twenty miles from central London. Two others (Chelsea College and Queen Elizabeth College) have merged (with effect from 1 August) with King's College to form an institution with more than 6,000 students (plus a medical school). The result is that the university's main teaching functions are for the first time concentrated in a handful of institutions, four large establishments (Imperial, King's, Queen Mary and University Colleges), Birkbeck College which has a special interest in part-time students, the London School of Economics and the amalgamation in the Surrey countryside. (One small college, Westfield, looks like sinking without trace unless it decides in the next few weeks to merge with the new King's College, to be known as KQC for the next five years.)

The objective of these upheavals was originally to save money, and that may eventually be possible. More immediately, however, the crying need is to find capital with which to make the planned amalgamations into effective teaching institutions. With the real funds at the disposal of the University Grants Committee shrinking by between one and two per cent a year, and with little prospect that the British government will dig into its pocket to help out just one university, much of the brave planning of the past few years may come to nothing. The most obvious danger for the colleges (and those who teach in them) is that the grants committee will be forced by sheer penury to shrink student numbers until the existing buildings will accommodate them.

The university is not well placed to defend itself. Administratively it is a mess. Although students at each of the teaching establishments are in theory members of the university as a whole, in practice the large institutions are separate and autonomous. Students are taught where they are recruited, and the different colleges distinguish carefully between each other's degrees. Financially, the colleges are supported by funds from the central federal university (but Imperial College deals separately with the grants committee on the grounds that it is a technological centre of national importance). Everybody agrees that the procedures for sharing out the funds are unsatisfactory, primarily because the customers are never helped to understand the principles on which decisions have been made. The system is also cumbersome; it is a standing joke that the University of London is usually about a year late in replying to the questionnaires that have been a standard part of university administration.

Left to itself, the university would probably collapse under its own weight in the years ahead. Now that the federal university's main teaching functions are no longer distributed among small as well as large institutions, the temptation to circumvent the university machinery, and to deal directly with the grants committee, will be strong. The result would be that the richness of the present diversity within the university would be lost. Most academics within the university know that to be the case, but there is also a limit to their willingness to put up with an administrative system which is not merely cumbersome but which lacks intellectual coherence. The challenge for the university's bosses and in particular for Lord Flowers, the head of Imperial College who takes over as vice-chancellor of the university next month, is that of deciding whether there is a way of keeping the university together and then of persuading those who work in it to follow such a course. It will be a struggle, but one worth making. □

Defence research

Defence to support UK academic research

IN a move that could more than double its financial support for university research over the next three years, the British Ministry of Defence (MOD) is embarking on two new collaborative schemes involving universities, industry and its own research establishments. The increase in funds, amounting to anything up to £15 million on top of the £9 million already spent annually on direct collaboration with universities, will come from MOD's research budget, now running at about £380 million per year.

More than one third of MOD's research budget is spent externally, most of it in industry on applied research with specific

applications in mind — not to be confused with the funds spent on development, which in total amount annually to £1,900 million. But a small percentage of the research money goes into strategic ("seed-corn") research. About five per cent of work carried out in the research establishments falls into this category. Externally, MOD supports some 650 research projects among 72 universities, colleges and polytechnics.

For some time, academics suffering a steady squeeze in funds for science have been casting covetous looks at MOD's resources, comparing, for example, its £380 million research budget with the £280 million available to the Science and Engineering Research Council (SERC). Partly in response to concern that it should generally seek to do more for the country's science base, but partly because of its own need to increase the return on its funds, MOD has set up a collaborative scheme for university research grants with several of the research councils, particularly SERC and the Natural Environmental Research Council (NERC).

The new scheme, to start towards the end of this year, should involve only minor change in the councils' grant funding procedures. Applicants will be invited to indicate whether they are interested in, or opposed to, part support by MOD. Following peer review, research staff in MOD will assess appropriate grant proposals and offer up to 50 per cent of the required support. The councils will then decide whether to provide the balance.

Both MOD and the research councils emphasize that the new scheme should not bend council funds towards defence-related research. And an MOD spokesman says that grants with significant defence relevance that fail to obtain council support may still be taken up by MOD. Furthermore, jointly funded research projects will be subject to restrictions on publication only when patents are involved or when researchers move into classified areas. Such occasions "are conceivable", according to the spokesman, "but we will bend over backwards to keep work unclassified. Our aim is not to be bureaucratic." Recent restrictions on US researchers by their Department of Defense, such as the much publicized cancellation of sessions at a recent physics conference, are viewed at MOD with disfavour.

MOD is to circulate every university with a portfolio explaining the scheme and listing the specific areas of primary interest together with names of principal MOD researchers so that interested academics can establish informal contacts. The research councils will issue announce-

ments of opportunity for grant applications, with a deadline of 1 December.

A second scheme for university collaboration is being set up by MOD that will involve the research councils only peripherally through their peer review panels. This scheme has been stimulated by MOD's experience of the Alvey scheme for university/industry collaboration in information technology, and will aim to stimulate such activity in other areas of defence-related research. MOD would support the whole of the university component, to the tune of £1 million in the first year increasing to about £4 million in 1988. By that time, MOD expects to spend £5 million annually on the collaborative grants scheme and £6 million direct to the universities. But if the demand is strong enough, a further £5 million could be available.

Phillip Campbell

University of Geneva

Illmensee's view

WITH the collaboration of others in his laboratory, Dr Karl Illmensee is still hoping to reproduce some of his early experiments on mice before he leaves the University of Geneva in September 1987. The difficulties in doing so, he said this week, are partly the result of continual interruptions caused by the need to attend to administrative affairs connected with the events that led him not to seek an extension of his contract with the University of Geneva (see *Nature* 11 July, p.98).

"The climate here is so hostile that there is no possibility to do research", says Illmensee, and "there is false information" in the internal report that was to have been submitted to the faculty meeting that would have considered extending his contract beyond September 1987.

Apart from interruptions from the university and the press, says Illmensee, the problems of repeating some of the past results are normal scientific difficulties. He has sufficient financial support from the university to attempt to reproduce past experiments and to continue with some of the research that has been interrupted, in close collaboration with others in his laboratory. These include three postdoctoral scientists, one from Hungary and two from West Germany, who have joined the laboratory in the past year.

"My priority", says Illmensee, "is to repeat the experiments of 1982 involving teratocarcinomas." The problem, he claims, is still that of re-establishing the teratocarcinomas. The second priority is to repeat the 1977 production of homozygous diploid mice using a technique that nobody has been able to reproduce. "I have no idea if the time (until September 1987) will be sufficient for me to complete these experiments", he says.

Peter Newmark

Genetic engineering patents

Interferon interference

LEGAL tussles over the commercialization of alpha-interferon have taken a new turn with the filing in Vienna by Biogen NV of a complaint against Boehringer Ingelheim Zentrale GmbH for its marketing of a product containing an alpha-interferon produced by recombinant DNA technology. Last August, Biogen was granted a broad European patent for "recombinant" alpha-interferons. Boehringer Ingelheim is one of several companies that have registered their opposition to the patent with the European Patent Office.

Biogen, of Geneva, Switzerland, and Cambridge, Massachusetts, is suing the West German pharmaceutical company Boehringer Ingelheim over an eyedrop for the treatment of keratitis caused by the herpes simplex virus that has been marketed in Austria since early this year. Acknowledging that the product contains a recombinant alpha-interferon, Dr Dieter Laudien, head of the Boehringer Ingelheim patent department, says that the company, and its subsidiary Bender, does not believe that Biogen's patent can be enforced.

Its opposition, lodged with the European Patent Office in May, is based mainly on evidence that enough information about alpha-interferon and its sequence was known and published before Biogen's patent filing to make Biogen's achievements "non-inventive". By starting its lawsuit against Boehringer Ingelheim, Biogen has clearly signalled that it is optimistic that its European patent will survive the opposition when the matter is considered, probably in a few months time. Meanwhile Biogen is still expecting to receive patent protection in the United States despite the rival patent granted to Hoffman-LaRoche (see *Nature* 21 March, p. 207).

Peter Newmark

Space business

Suppliers ready to break rules

Washington

THE Office of Technology Assessment (OTA) of the US Congress has paid the international space industry the compliment of an economic analysis of its prospects not very different from what might be written about an established industry. In a report published this week, *International Cooperation and Competition in Civilian Space Activities*, OTA says that attempts at international collaboration will probably be blocked by national policies, but recommends that, wherever possible, governments should let space industries develop along unregulated lines.

Much of the report is meant to alert US industry to the emergence of competition in some space activities in other industrialized countries to which, the report says, US policies have not fully adapted. The report identifies the members of the European Space Agency (France in particular) and also Japan as the chief sources of competition during the coming decade.

Space activities are still predominantly within the domain of government, according to OTA. And the big spenders are the United States (which spent \$17,500 million in 1984 on civil and military applications) and the Soviet Union (which is said to have 600,000 people working in the field). US spending is 20 times that of the European Space Agency (ESA), and is the equivalent of 0.47 per cent of gross national product (GNP) compared with 0.08 per cent for France, the next in line.

OTA thinks it unlikely that there will emerge a competitive environment for trade in space-related services and products. "Since France and Japan... have made the decision to join the United States as space powers, it would be wishful thinking to believe that they will fully abide by the trade rules.."

Even within the United States, OTA is not convinced that there will be enough business to tempt private companies into the provision of launching vehicles, as three aerospace companies (Transpace carriers, General Dynamics and Martin Marietta) are in principle equipped to do. The report also says that the foreseeable demand for launching facilities is what it is at present — the need to put communications satellites in orbit — and that other satellite uses, such as for remote sensing and materials processing, "are even more uncertain".

The volume of demand should nevertheless be enough to keep both the US shuttle and Ariane fully occupied. According to figures compiled by Rockwell International and quoted by OTA, the combination of civil and military needs in the United States would be enough to occupy 24 shuttle flights a year by 1988, with possible demand making necessary a further 20 flights a year in the succeeding

five years. These calculations assume that Ariane would be making 10 flights a year from now on.

If these estimates are correct, OTA says, there may be a need for extra launching capacity by 1988. But if the US defence needs are in part met by some other launching mechanism, either the shuttle or Ariane could be under-occupied between then and 1994. Those are the circumstances in which competition might occur.

At what price? The cost of a dedicated shuttle flight has already increased from the \$18 million estimated in 1975 to something like \$80 million now, but OTA points out that this is considerably less than the real cost of the five shuttle flights in 1983, which are estimated to have cost \$375 million each. By 1988, when overhead costs will be spread over more launches, it may still be necessary to charge more than \$100 million for a dedicated launch.

At these rates, OTA says, the shuttle should still be cheaper than Ariane as a launching vehicle for geosynchronous satellites (which is reckoned to amount to \$20,000 for each pound of mass in orbit). But competition may be hindered by the terms on which launching services are offered; the US National Aeronautics and

Space Administration (NASA) is required to ask for all the cost in advance, but Ariane's managers take graduated payments.

OTA offers the US government a series of policy choices on the shuttle. If, the report says, the objective is to capture a large percentage of the commercial demand for satellite launchings, the result will probably be increased demand for use of the shuttle, perhaps even a need for more orbiting vehicles, and increased cost. If the US government were deliberately to encourage private US companies to enter the business, they would probably compete successfully with Ariane, but NASA's role as a space carrier would be reduced. The report also points out that a strategy intended to make the shuttle the vehicle to meet "all current and future" needs in the United States would greatly increase the cost of the shuttle programme and probably put Ariane out of business.

On the business of building communications satellites, OTA argues that US suppliers are the cheapest source of hardware (in spite of the strong dollar) and were able to compete successfully for the contracts to build all 20 of the Intelsat consortium satellites to be launched in the period 1984-89. But OTA notes that all 19 European satellites to be launched for European owners during the same period will be built in Europe, as will be the nine planned Japanese satellites to be built in Japan. □

US pharmaceuticals

Monsanto and Searle to merge

Washington

THE planned merger of Monsanto and G.D. Searle, announced last week, will provide a quick shot in the arm for Monsanto's growing interest in drugs for human disease. The move will also give Monsanto access to a distribution network for pharmaceutical products, the development of which is one of the objectives of the recently expanded research facility at the company headquarters at St Louis, Missouri.

Although the Searle family, which at present owns 20 per cent of the stock in G.D. Searle, has made no secret of its wish to diversify its interest since last September, the planned merger appears to have taken the pharmaceutical industry by surprise. The plan is that Monsanto should offer to buy all the outstanding shares in G.D. Searle for a total cost of \$2,700 million.

The combined operation will have total sales of \$8,000 million a year, with Searle rather less than 20 per cent the size of Monsanto. Both businesses have substantial interests very different from their point of overlap in biotechnology, which appears to have been one of the chief reasons for the merger. Monsanto is primarily a manufacturer of plastics and agri-

cultural and general chemicals, while Searle in the past two years has become, at least temporarily, the predominant supplier of the artificial sweeteners based on the di-amino acid aspartame.

Both companies have taken a close interest in biotechnology in the past few years. Searle, which has three major laboratories worldwide, has built up the laboratory at High Wycombe in Britain (with a complement of 600) to be a major centre of research in recombinant-DNA techniques. Monsanto's directly comparable interest is the somewhat smaller unit based at its Life Sciences Center at St Louis, opened formally only last year.

A spokesman for Searle said this week that the two research enterprises were likely only to reinforce each other. With the St Louis laboratory at the beginning of what is likely to be a period of growth and consolidation, the High Wycombe laboratory could well have the advantage of possession of the ground in any reorganization that comes about. But, formally, nothing can be done until Monsanto has made its formal offer for the shares of G.D. Searle, won the acceptance of a majority of the holders and then decided where it will raise the funds with which to pay for them.

John Maddox

Academic innovation

Cambridge science park succeeds

LIKE a well-focused laser beam, the Cambridge Science Park (CSP) is hot in the middle. Its heat is that of fast growth — witness the new construction in every direction, the rising profit margins of the companies and the traffic jams outside the park.

This growth of high technology in a previously "backwater" university town has inspired a flurry of analysis about the reproducibility of the CSP experience and its long-term growth. How fertile is the industry/university link for jobs and increased income? Cambridge has been compared to the early Silicon Valley; does it also need the anchor of big companies with a marketing mentality gauged to the mass market rather than to the specialized niche?

CSP, with its 40-odd companies and 1,000 employees, is only a small part of what is now popularly called the Cambridge phenomenon. Since the early 1960s, some high-technology companies employing 14,000 people have been established in Cambridge, most of them with direct links to the university. CSP's acknowledged role is as symbol, proof and drawing card for what is happening in Cambridge. It is not, however, simply a further extension of that activity. Located just outside the town on land owned by Trinity College, CSP has high-rent buildings, lots of open space, a lake complete with rather antagonistic geese and its own personality.

The scarcity of good industrial sites in Cambridge means that CSP is one of the best places in the area. For this reason it attracts not only Cambridge companies that are growing or moving upmarket but also companies from outside the region which come because of Cambridge's reputation and because Britain's oldest science park is now a good address. They come to get away from "metal-bashing" industrial centres to a town where the air is clean. And increasingly they come to CSP because of its accumulated experience in getting new technology into established industry.

Many CSP companies such as Laser-Scan Laboratories, with its laser-deflection display devices for location of aircraft in distress, use computer technology; the hardware/software companies themselves, however, have so far chosen to stay in town, close to the university computer facilities. Spin-off companies are common among these, as they are in Silicon Valley and on Boston's Route 128. Because of CSP's small size and high specialization, there is less interaction among companies at the park.

An important factor in CSP's growing success is the unconventionality of the "oddball" entrepreneurs. Ideas that might previously never have seen the light of day

outside an academic journal are now not only taking reproducible form but are also making money. At one turn, for example, Dr Felix "Polywater" Franks has turned his expertise to water in supercooling cells. At present, genetic engineers and companies doing drug tests on cell lines freeze their cells to preserve them. "Cells are contrary beasts", says Dr Franks; even if they can be frozen, they are often killed or experience genetic drift. Supercooling, according to Franks, keeps the cells alive and pure.

Another innovator is Andrew Goodfellow of Goodfellow Metals, which specializes in thin films and in changing the surface properties of metals by ion implantation. Goodfellow commercialized one of the first Cambridge inventions to become a success — the Huxley-Goodfellow micromanipulator originally designed by Sir Andrew Huxley. Britain, according to Mr Goodfellow, "breeds a nation of losers". The most important difference at Cambridge is the attitude of mind fostered there.

Dr William Bolton, of Cambridge Robotics, runs a tiny company involved in dedicated electronics for foundries. He sees his role as educating both engineering graduates and foundry management, showing the former that the problems of the assembly line can be challenging and providing the latter with graduates already experienced in technical and financial issues.

The Microelectronics Research Laboratory is so far an anomaly at CSP because it is a university facility. Many of the projects the laboratory works on, according to the director Dr H. Ahmed, are of direct benefit to industry. Examples include research on electron and ion beams, as well as new ways of putting silicon on top of insulators. The laboratory does not do contract research but, instead, collaborates with interested companies, a sharing that often results in joint publications.

Trinity's role as owner of the CSP land places it in a special relationship to the occupants of the park. The juxtaposition of high-technology companies with a college known for scientific excellence might be expected. On the other hand, this marriage of interests is an oxymoron: the universities in Britain have traditionally had nothing to do with applied research, and the timescale of Cambridge, "where things are happening fast if they are promised by next term", is antipathetic to the aggressive speed of high technology.

This timescale has its advantages. Dr J. Bradfield, senior bursar of Trinity College, says that growth was slow before 1980, but because Trinity owned the land already, looked at projects in terms of decades if not centuries, and had no speci-

fic plans for CSP, the park was never in danger of being labelled a failure. In addition, though Trinity definitely wants a close relationship between the CSP industries and the university, it sees no reason to force or formalize them. Here is the crux of the industry/university bridge; according to Bradfield, it is the "accidental and unexpected friends" that provide the finest spark.

Trinity's support of CSP has been an important catalyst for the entire Cambridge phenomenon, lending confidence to the movement according to Dr Nick Segal, whose firm Segal Quince & Partners recently published a report on Cambridge high technology. Dr Bradfield points to the 1960s debate over the role of the university and Cambridge's image as unindustrial (as opposed to Oxford) and quaint. Now, according to Dr Bradfield, CSP has shown that applied research can achieve "results of great interest that also make money".

Acceptance for the changing dynamic of university research and industry is expressed in many different ways. According to Dr Peter Woodsford of Laser-Scan, a scant five years ago his company was not allowed to specify the kind of work a Cavendish research fellow that it funded would pursue. He believes he would have such freedom now.

CSP's growth curve, says Dr Bradfield, is virtually classic in form; a slow first few years followed by a steep rise. CSP insiders agree that CSP, along with the entire Cambridge phenomenon, is still far from reaching a plateau.

As for second-stage growth of the Cambridge phenomenon, one issue is how many small companies will be bought out — a trend already begun between US companies and CAD firms in town. Dr S. Bragg is head of the Wolfson Industrial Unit, whose business is providing bridges between industry and university. He predicts that small enterprises will continue to be started, but cannot say whether existing companies will grow suddenly or stay the same size.

CSP's success will be a function of the model analysts apply. There is no reason why a large number of small companies should not provide at least a certain volume of employment and income, at the same time retaining flexibility by constantly bubbling new ideas to the surface. Failing companies would be quickly replaced. It is tempting, however, to apply another model — the leaven of large firms governed by a marketing mentality that thinks in billions, not millions — because this model applies to the older larger science parks in the United States. Large companies offer widespread use of products, lots of jobs and the promise of stability. Already there is an IBM "listening post" on CSP. But for many of the innovators there, big fish moving into their pond would not be a welcome transformation.

Elizabeth Collins

UK laboratory animals

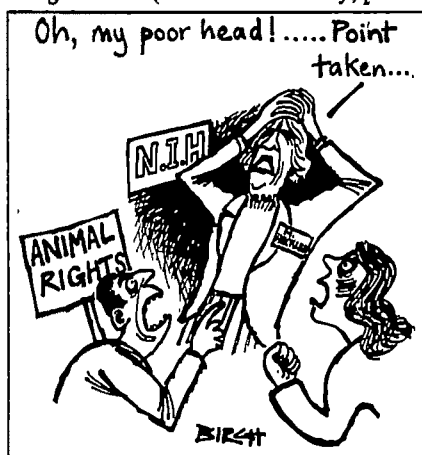
Experimentation reduced

THERE are signs that the pressure of public opinion has caused researchers to think more carefully about experiments on living animals. The number of such experiments in Britain decreased by 4 per cent last year. And although the number of licensed experiments has remained constant, the 3.5 million new experiments reported in 1984 is the smallest since 1959, according to statistics published by the Home Office in its annual report last week (Cmd 9570, HMSO, £4.70). Just over half of the licences to perform experiments on living animals are held by people in universities and about a quarter by those working for commercial companies; but 53 per cent of the experiments are performed now in the commercial sector and only 22 per cent in universities.

The largest decrease between 1983 and 1984, occurred in experiments on amphibians and reptiles (a 44 per cent fall) and

mice (8 per cent down). Experiments on birds and fish increased but, even here, the total is still less than that in 1980. Even cosmetics testing has been reduced, although only by 3 per cent of the amount in 1983. Of the experiments performed on live animals in Britain, almost all (80 per cent) are either to investigate safety and hazards or to look at body structure and function.

The emphasis on pain placed in the recent white paper (policy document) (Cmd 9521) on *Scientific procedures on living animals* (see *Nature* 23 May, p. 267)



is continued in the Home Office annual report. The Cruelty to Animals Act requires that animals must be anaesthetized during an entire experiment and, if the animal is likely to be affected post-operatively, killed before recovery. If this frustrates the object of the experiment, the researcher must obtain a special licence from the Home Secretary signed by the president of a learned society and a professor of medical science, as well as reporting each year on details of the experiments performed. The act only allows experiments at all for the purposes of increasing physiological knowledge or for prolonging life and alleviating suffering.

The white paper, which seeks to restrict animal experiments further, is unlikely to be debated in parliament in the next 12 months. This delay has disappointed organizations such as the British Veterinary Association, the Committee for the Reform of Animal Experimentation and the Fund for the Replacement of Animals in Medical Experiments. They feel that the white paper proposals are a necessary first step in the protection of laboratory animals and ensuring that their suffering is minimized. But the government is reluctant to set a timetable for debating such a controversial issue.

Maxine Clarke

• The 21 member states of the Council of Europe have adopted a convention that will lead to better protection of vertebrate animals used for scientific purposes. Bri-

tain will sign the convention "in the early autumn", Home Secretary Mr Leon Brittan says. Britain has played a leading role in drafting the convention and the government intends to use an article "enabling member states to adopt more restrictive measures than those laid down". The convention aims "to balance the needs of research with the consideration owing to live animals; to protect them from pain and suffering; and to ensure that non-sentient alternatives are used wherever practicable". Member states will be required to publish annually statistics on the use of animals. □

Framatome

French company still adrift

FRAMATOME, the French constructor of pressure vessels for the French nuclear reactor network, is still looking for a buyer a year after the collapse of its parent company, the steelmakers Creusot-Loire.

The reason for the delay is simply that Framatome at present does not appear a good buy. Despite the French nuclear success story, most of the reactors that France needs are already built or under construction. The government has now reduced orders to one 1,300-MW reactor every couple of years, and there is unlikely to be an upturn in orders before the oldest reactors, built in the mid-1970s, need replacement in the late 1990s. Exports of French pressurized water reactors (PWRs) have also proved almost impossible. And while Framatome will supply the pressure vessel for the first British PWR at Sizewell, if planning consent follows the public inquiry report due at the end of this year, the French fear that the British market may later be closed. British constructors are expected to get together with Westinghouse to design their own version for the world market, with the result that even if there is an expansion of PWR construction in Britain after Sizewell, Framatome may not benefit. And even if Framatome does get a place, the prospect seems far enough off that the company's immediate problems will not thereby be solved.

Thus the French atomic energy commission, which is now looking after Framatome, would like the company to diversify. Uses for nuclear pressure vessels are, however, relatively few; and Framatome staff are already beginning to fear Framatome's dismantlement. That, however, might prove to be such a political embarrassment for the present government, with nuclear power being rightly seen in France as a national success story, that it can be discounted for the time being. If, however, the right is returned to power at the next elections, it would be a simple stroke to dismantle Framatome — and blame it on the economic failures of the previous government. Robert Walgate

US animal rights

Washington

A FOUR-day sit-in by animal rights protesters at the National Institutes of Health (NIH) ended late last week with the surprise announcement by Secretary of Health and Human Services Margaret Heckler that she was meeting the demonstrators' demand for an end to NIH support of the University of Pennsylvania's Head Injury Laboratory.

The laboratory was the target last year of a break-in by the Animal Liberation Front, during which equipment was vandalized and videotapes of experiments were stolen. Researchers at the laboratory induce brain injuries in monkeys to study how damage and recovery occurs in head-injury accidents.

According to an NIH spokesman, NIH officials were already investigating complaints about violations of its animal care regulations at the laboratory when the sit-in began; the investigators' report was expedited by one day because of the protest, however.

The NIH investigators, who reviewed videotapes and inspected the laboratory, concluded that the researchers had not properly trained or supervised technicians, had performed surgery on animals in unsterile environments and had failed to use proper anaesthesia and analgesia for the animals to minimize pain. NIH left open the possibility that financial support could be reinstated. NIH director James Wyngaarden said he would make a final decision after the University of Pennsylvania has been given a chance to respond. NIH did not criticize *per se* the use of monkeys in the experiments or the experimental protocols.

Stephen Budiansky

French information

New centre for Nancy

Not before time, France is to reorganize its whole system for managing and distributing scientific information, the French government announced last week, thus responding to a long campaign by the Centre National de la Recherche Scientifique (CNRS), the principal research council, to bring the nation's libraries together in one great documentation system.

According to M. Goéry Delacote, director of scientific and technical information at CNRS, one target will be to catch up with the British Library Lending Division (BLLD), which is now central-

ized at Boston Spa in Yorkshire. Fifteen years ago, said Delacote, the CNRS documentation centre (CDST) and BLLD serviced 300,000 requests each year for photocopies of papers and other items in the sciences. Today, BLLD's computerized systems deliver 2,700,000 a year, while CDST's figure has not changed.

Something had to be done to improve the distribution of papers, a problem exacerbated by the poor financial condition of French university libraries. An opportunity came last year when the government offered to relocate the 350-staff CDST, now in Paris, in the depressed steel town of Nancy. Since many of the staff will probably choose to stay in Paris, this was an offer of jobs to Nancy, and of increased space to CDST.

There will be a new institution, the Agence Nationale de l'Information Scientifique et Technique (ANSTI), whose job will be to coordinate not only CDST but also the many university libraries, the information systems of the medical research council (INSERM), the national collection of periodical catalogues (CCNP) which centralizes the journal lists of 2,300 French libraries, and other sources into one system which should look, as far as possible, simple and unified to the potential user.

According to Delacote, the aim will be not only to increase the volume of papers transported and transmitted in France but also to increase the speed of the various services from their present "rather long" average of 10 days from request to delivery to something more like one or two. "We also want the system to be as comprehensive as possible", said Delacote, so that "95 per cent of the time" a user will find that the paper requested is available.

ANSTI is expected to recommend that the new CDST centre at Nancy will provide the basic document delivery system, will host and develop the French abstracting database Pascal (for the hard sciences) and Francis (for the humanities and social sciences), and provide a centre for information management.

And while France may appear to be coming from behind in this area, it is also moving rapidly into the overtaking lane. Delacote believes a scientific information system like CDST must invest "10, 15 even 20" per cent of its budget in research, and he has put FF25 million (£2 million) of his budget into a project for automatic document delivery dubbed "Transdoc". This experimental electronic archiving and access system is comparing optical digital disks and a microfiche archive run by a computer, and should produce a clear choice of high-volume archiving system "by the end of this year". In this France is running "parallel to or even ahead of US systems".

Robert Walgate

Jürgen Neffe

More pork-barrel politics

Washington

DESPITE condemnation by the Association of American Universities, the National Academy of Sciences and the National Science Board, direct appeals to legislators for funding of academic research facilities are continuing, and continuing to pay off. This year, once again, a number of construction grants to universities were approved on the floor of the House of Representatives and the Senate, virtually without debate; the grants had not been submitted to funding agencies previously or subjected to scientific peer review of any kind.

Topping the list this year is Dartmouth College, which, if the House agrees to the Senate's version of the 1985 supplemental appropriations bill, will receive a \$15 million grant for construction projects at its engineering school.

The grant was approved by the Senate without a vote on an amendment offered by Senator Warren Rudman, who represents the state of New Hampshire, where Dartmouth is located. That same amendment also provided \$10 million for a "south wing rehabilitation project" at the Organ Health Science University, located in the home state of Senator Bob Packwood, the chairman of the Senate Appropriations Committee.

Senator Orrin Hatch (Republican, Utah) introduced two amendments to provide support for a \$6 million cancer screening and research centre in St George, Utah, and a centre for the study of health effects of new energy technologies at the University of Utah.

Both projects had been authorized last year in amendments to the Head Start reauthorization bill which Hatch was able to push through in his position as chairman of the Labor and Human Resources Committee. Head Start is a programme that provides special tutoring to disadvantaged children.

Senator Robert Dole (Republican, Kansas), the Senate's majority leader, secured \$200,000 for a remote-sensing research project at the University of Kansas. The one contribution from the Democratic minority in the Senate was a \$500,000 amendment proposed on behalf of Senator Edward Kennedy of Massachusetts for a Chinese-US student exchange programme at Tufts University in Boston.

Earlier this year, Senator Alfonse D'Amato (Republican, New York) pushed through a measure to set aside \$1 million for a computer research programme at the University of Syracuse; the money comes out of a \$25 million university research initiative at the Department of Defense that was to be used entirely for investigator-initiated, peer-reviewed grants for basic research.

Stephen Budiansky

West Germany

Support for biotechnology

Hamburg

By providing DM 1,000 million between 1985 and 1989, the Minister for Research and Technology, Heinz Riesenhuber (CDU), hopes to enable West Germany's biotechnological research to perform "jumps of perception" as chemical research did 150 years ago.

One principal task is to expand the Gesellschaft für Biotechnologische Forschung (GBF) into a national centre of biotechnology. Another is to promote the gene-centres in Cologne (plant cultivation), Heidelberg (immune defence, antibodies for cancer and rheumatism diagnosis) and Munich (chemical synthesis of nucleic acids) and to support two new centres for biotechnological process engineering.

Apart from financing basic research, Dr Riesenhuber, generally known as an obliging supporter of industrial research, plans to spend DM 120 million on a programme to further the biotechnological industry (*Programm zur Förderung der biotechnologischen Industrie*). Up to 40 per cent of expenses for projects will be refundable by the ministry up to a maximum of DM 600,000. This programme covers developments in cell and tissue culture techniques, genetic engineering, biotechnical procedures with human, animal and plant cells or manipulated microorganisms, new biological systems of plant protection and the use of enzymes in medical and food research.

Development of new apparatus and devices for biotechnological production will also be promoted in the new programme. Dr Riesenhuber is optimistic about the prospects.

The total value of biotechnological products in West Germany came to DM 17 million in 1983, compared with an estimated DM 40,000 million worldwide.

Bhopal

Birth defect fears allayed

New Delhi

ALTHOUGH there was an increase in the number of abortions, the gas leak tragedy in Bhopal last December did not result in the birth of handicapped babies as had been feared. Some 3,000 women were thought to be pregnant when methyl isocyanate (MIC) leaked from the Union Carbide pesticide factory on 2 December 1984, killing over 2,000 people. The Indian Council of Medical Research (ICMR), which has been following up 1,500 cases of pregnancy, says that by June, 500 babies had been born. Of these, five were born dead and three had congenital defects, an incidence statistically no higher than in a normal population. Most babies were low in birth weight (2 kg) but this too is not uncommon in India.

The rate of 17 abortions for 100 pregnancies was, however, above the national average. Dr V. Ramalingaswami, head of ICMR, says that this was not unexpected and that abortions increase by as much as four times during famine or epidemics, and the Bhopal tragedy was a comparable situation.

Soon after the accident, the state government of Madhya Pradesh considered compulsory termination of all pregnancies in the affected population. The proposal was dropped on the advice of ICMR which had no scientific data to warrant this step.

ICMR will, however, watch for any abnormality in the 1,000 babies expected to be born in the next two months from mothers who were in their first trimester of pregnancy at the time of the accident. "If MIC was really toxic to the fetus, it will be this group that needs watching", Dr Ramalingaswami said. According to his deputy, Dr S. Sriramachari, scattered anomalies in different organs would be a sure sign of MIC effect. But on the evidence so far, ICMR does not expect to find any anomalous births. In women yet to be delivered, ultrasonic scanning has shown fetal growth retardation in 15 per cent of cases, not unusual in India.

Meanwhile, ICMR has completed a health survey of 90,000 people, half the population that was exposed to MIC. Most are still undergoing treatments for respiratory and asthma-like problems, weakness of limbs and inability to carry on their usual work. Patients who had apparently recovered are said to be returning to clinics for recurring ailments. Some 1,200 patients are receiving injections of sodium thiosulphate (STS) which, according to Sriramachari, has been effective. STS is an antidote to cyanide poisoning but no one is sure why it works in MIC victims. It is possible that some MIC broke up into hydrogen cyanide when it escaped from the factory or that it produced cyanide radicals inside the body, after being inhaled. In any case,

ICMR's follow-up of STS-treated cases had revealed that STS mopped up the cyanide from the body and excreted it through the urine. ICMR is not sure how long to keep the patients on STS as they are found to develop symptoms once the injections are withdrawn.

While there has been no breakthrough in treatment, researchers claim to have obtained new information on the mechan-

ism of MIC poisoning. According to Sriramachari, analysis of blood samples from 60 cases has clearly shown that MIC attaches "to the terminal amino group of the alpha chain of the globulin part of the haemoglobin by a process of carbamylation".

This mechanism, he says, "explains the observed clinical features such as respiratory distress, associated with cherry-red venous blood, and toxicity due to failure of utilization and removal of CO₂ and consequently, deoxygenation".

K.S. Jayaraman

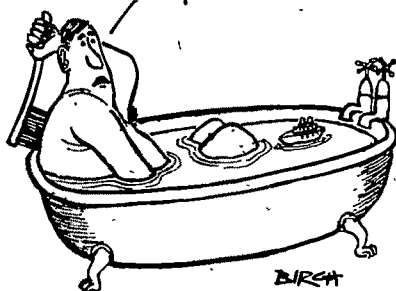
Eureka

Party launches agenda only

EUREKA, the French-led programme to unite European high-technology industry behind a handful of new products from driverless tractors to supercomputers, came into official existence on Wednesday last week. But exactly what Eureka is remains obscure, a situation that led the Paris newspaper *Le Monde* to comment that the decision was like film-maker Woody Allen's remark, "the answer is yes, but exactly what was the question?"

Nevertheless, officials present at the Paris meeting of ministers from 16 European states and the European commissioner for research count the broad agreement on Eureka a success. They point out that in the afternoon and evening allotted

We'll think of what it is soon...



to the discussion, there was hardly time for each delegation to make its own position statement, let alone to reach agreement on "points of detail".

Among the details not yet settled are the fundamental issues of who pays, how the programme is to be managed and on exactly what criteria projects are to be selected. The French President, M. François Mitterrand, put FF1,000 million (£82 million) on the table for 1986. West Germany made "ambiguous" noises about finding DM300 million (£75 million). But otherwise, there was silence from the 17 delegations.

There was, however, a frosty joke between Jacques Delors, president of the European Commission, and Roland Dumas, the French foreign minister. Delors, who has been put out by French insistence that Eureka should not fall into the hands of the "bureaucratic" Commission, has an ambitious technology programme of his own, and said he would

dearly like 20 per cent of national research and development budgets, to run joint European projects. Dumas replied that he would dearly like 20 per cent of the Commission's budget, to support Eureka. Stalemate.

Predictably, Britain was adamant that no special funding was required. Foreign Minister Geoffrey Howe claimed that Europe spends more on research and development than Japan, but gets less out of it. Therefore the problem was not one of research budgets but rather of opening markets. He therefore suggested, as part of a British package of projects for Eureka, that any products created through Eureka collaborations should receive what he described as "Eurotype" certificates — which should guarantee the product free passage through the many restrictive trade barriers that exist in Europe. This proposal is said to have been well received.

For now, the management structure of Eureka remains an open question, despite a strong play by France for a "small" office to be set up in Paris. Dumas said after the meeting that "some countries" had asked France to host the Eureka secretariat, but for the moment Eureka remains without a home.

In the brief final communiqué from Paris, the European Commission is described as acting "in strict consultation" with the Eureka action group. The communiqué also says that Delors' proposals "adopted" by the recent summit meeting in Milan will be "put into action", but what exactly this means will become clear only in future European Council meetings, but it must be doubted whether it will go anywhere near as far as the doubling of Brussels research budgets that has long been in the Commission's mind.

Otherwise, the communiqué says that "particular importance" will now be attached to "encouraging and stimulating the formulation of concrete projects among the industries and research centres of Europe", and to "conceiving the appropriate financial mechanisms". That is what everyone, industry included, is waiting for.

Robert Walgate

Poland

Environment at long-term risk

POLAND will need at least two decades of "huge social efforts and financial outlay" to restore an ecological balance that will satisfy the nation, according to Stefan Jarzebski, head of the Office of Environmental Protection and Water Economy. Presenting a report on the state of the environment over the past five years, Jarzebski told the Sejm (Parliament) last month that the losses caused by the degradation of the environment are now considerably greater than what is spent on attempting to counteract them. Decades of neglect of the environment have produced "calculable economic losses" — smaller crops, smaller yields of forest products, higher costs of water treatment for industry and domestic use.

A state inspectorate of environmental protection established in January 1980 has been insufficient, as the Sejm debate and resolution on the report made clear, to halt the deterioration of the environment. Since the law came into force, "extraordinary threats" to the environment have almost quadrupled, from 396 incidents in 1981 to 1,395 in 1984. Factories find it cheaper to pay fines for emitting pollutants than to take steps to eliminate the sources of pollution. (In 1984, almost 3,000 enterprises were fined a total of more than 2,500 million zloty.) Many important anti-pollution measures recommended under the 1980 law could not be put into practice, or even started, the Sejm noted, presumably as a result of the economic crisis. It should be noted, incidentally, that the closing of the Skawina aluminium refinery near Kraków in January 1981, an achievement to which Jarzebski attached great importance in his report, was not a government initiative at all. Demands to close the plant (which was discharging hydrogen fluoride into the atmosphere) came from the Kraków Ecological Club, one of the many private initiatives which sprang up in the wake of Solidarity, and which, with the backing of the Archbishop of Kraków, Cardinal Franciszek Macharski, managed to convince the relevant authorities that the cost of keeping the plant open, in terms of damage to the local environment and to the health of the population, was greater than the loss to the economy produced by closing it.

In spite of the closure of the Skawina plant, the Kraków area still remains one of Poland's black spots, the others being the Upper Silesian industrial belt (centred on Katowice), the Legnica-Głogów copper belt and the Gdańsk and Puck gulfs. Here, the Sejm decided, the deterioration may be classed as "ecological calamity". Twenty-three areas were designated as being at ecological risk. Even in the capital, Warsaw, householders have to boil their drinking water, and, in spite of this

precautionary measure, digestive disorders, urinary tract illness and cancer are on the increase.

The law of January 1980 was one of the few positive responses to environmental problems of the Gierek regime which tended to deal with such matters by censoring public discussion of them. The present government seems at least prepared to discuss them, perhaps because it has a ready-made excuse in Poland's continuing economic problems for any failure of delay in taking action. The Sejm's response to Jarzebski's data was to call for a national programme on the protection of the environment up to the year 2010, a plan which is expected to take at least two years to complete. In the meantime, "undertakings serving protection of the environ-

ment", says the Sejm, should be written into the 1986–90 Five Year Plan.

How far these recommendations will be accepted and implemented is, of course, a matter for the government and the industrial managers.

● Three leading Polish dissidents, Jacek Kuroń, Adam Michnik and Tadeusz Jędrzak suggested, last February, that independent Committees for the Protection of the Environment of Silesia should be established, the underground newsletter *Przegląd Wiadomości Agencyjnych* reported on 2 June. The report drew special attention to the high child death rate from cancer in Silesia due to the pollution of the environment, but noted that the majority of the population seemed unwilling to face the reality of the threat. No concrete action seems to have been taken on the proposal, and Michnik is now serving a prison sentence and Jędrzak awaiting trial on political charges.

Vera Rich

Soil Survey

Budget squeeze hurts projects

THE dwindling staff of the Soil Survey of England and Wales is still in the dark about its future. The unit, based at the Rothamsted Agricultural Station, will nevertheless have to decide by the end of October how many members of its staff will be retained beyond the beginning of the next financial year on 1 April 1986, when its budget will be cut in half.

The problems at the Soil Survey appear to be an almost classic illustration of how the Rothschild principle for research support can turn sour. The survey, formally established in 1939, and now constitutionally a part of the British Agricultural and Food Research Council (AFRC), has been supported since 1972 by research commissioned from the Ministry of Agriculture, Fisheries and Food, most recently (in 1983–84) to the tune of £1.8 million a year. In January this year, however, the ministry let it be known that the annual grant would be cut in half (to £850,000) in the year beginning next April. Nothing has been proposed for the succeeding year.

One inevitable consequence of this uncertainty is that the survey is losing staff. By the beginning of this year, largely because of declining research commissions, the staff at the survey had shrunk to 70 (from a peak of 85 in 1976); now it has fallen to 64, largely by allowing vacancies to go unfilled.

The decision about next year's budget was announced by Lord Belstead, an agriculture minister, in the House of Lords on 29 January, who explained that the objective of the reduced grant from next year was the British government's belief that "this is an area which ought to be commissioned by those who use the work". Much in that spirit, AFRC has commissioned a consultants' study of the opportunities for

persuading the Soil Survey's users to pay for the work it does. The report of that study, not yet formally presented, is understood to take the view that the survey could become self-supporting only on a smaller scale, but that a full 50 per cent reduction would not be needed.

The Soil Survey appears thus to be one of the first casualties of the work of the Agricultural Priorities Board, appointed a year ago to replace the previous Joint Consultative Organisation for agricultural research. Lord Belstead said last January that the decision to cut the budget of the Soil Survey had been taken after consultation with the board.

In the past few months, some outside contract work has come the way of the Soil Survey, which says it has been able to raise £175,000 to support certain aspects of its work. "That's six people's jobs for a year", one staff member said. Meanwhile, the pattern of the survey's work has been modified so as to concentrate on projects that could be rounded off by next year. For the time being, work on the 1:50,000 soil map of England and Wales, a project planned to be completed only in the 1990s, has been put on the back burner.

In principle, according to ministry sources, there is no reason why AFRC should not shoulder some of the burden of keeping the Soil Survey alive, although the council itself is under serious pressure from a reduction of its budget. The most likely help from the survey is some adjustment of the cost of premises at Rothamsted, now valued at some £350,000 a year. Paradoxically, nothing has yet been said about the comparable soil survey operation in Scotland, based at the Macaulay Institute, which was reduced by a fifth in 1982 but whose more distant fate will not be decided until the end of August. □

Pain and laboratory animals

SIR — The government's proposals to update the Cruelty to Animals Act 1876 (*Nature* 23 May, p.267) have been widely reported as tightening up the present deplorable situation, a view we strongly challenge.

On the question of pain, the government has proposed that the "severity" of an experiment should be balanced against its potential benefit. Three categories of severity are envisaged: mild, moderate and substantial. In addition, the government has set a "top severity limit" where "an animal which is in severe pain or distress which cannot be alleviated shall be immediately and painlessly killed".

Since it is impossible to devise a method of measuring pain in animals it follows that what may be considered "severe" pain or distress by one person may be dismissed as mild, moderate or substantial by another. Some government departments and officials share our view on this matter. For instance, the Home Office Departmental Committee of Inquiry on Experiments on Animals 1965 (the Littlewood Committee) concluded that "it is not as a rule possible to assess degrees of real pain in animals" (para. 187) and "it is often much more difficult to detect physical pain in an animal than in a human patient" (para. 174).

The subjective assessment of "severe" pain or distress renders such pain conditions meaningless to the animals, while at the same time allowing the government

and experimenters to pretend that there are permissible levels of pain, and that animals are thereby protected against excessive suffering. Furthermore, how is the importance of the experiment to be defined and does this refer to therapeutic, commercial or scientific considerations? Take for example the LD50 test in which animals are deliberately poisoned to death, which is clearly one of the most painful animal experiments. On the basis of the government's proposals, LD50 would have to fulfil a really important need, since the test causes so much suffering. But LD50 is generally recognized as bad science, so would the government refuse to allow the test to be performed? Or would the "severity" of LD50s be redefined as "moderate" or "mild" to overcome these difficulties? The latter course seems highly probable since the government has stated that it will not prohibit the LD50 test.

But the idea of prohibiting experiments, such as the use of animals to gain manual dexterity, is already established in the Cruelty to Animals Act 1876 and could readily be extended to other types of experiments, as a positive step towards a completely ethical system of research and health care.

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Romanian reactors

SIR—We would like to provide some additional information on the Romanian nuclear programme (*Nature* 2 May, p.4). There is nothing misleading, as you put it, about our references to two plants now under construction in Romania.

The reactor civil works are well advanced on the first three units, the main excavation for the fourth unit has been started and site clearing has been commenced for the fifth. The office building and some of the permanent service buildings have already been completed and partly occupied.

The cooling water intake, pumping house is well advanced and the turbine foundations are reaching completion for the first and second units. The pipe fabrication shop, welder training and electrician shops are already in position.

In terms of manufacture, all the major orders have been placed. Babcock and Wilcox has already shipped heat exchangers and the first calandria or reactor vessel, the largest single component, is scheduled to be shipped from Canada before the end of this year. The arrangements between Atomic Energy of Canada Ltd (AECL) and the Romanians are that AECL will provide licence information so

that the Romanians can undertake a significant amount of the work themselves on the subsequent units.

The drawings and specifications have been delivered to the Romanians and they have a large engineering and drafting team of around 1,000 key staff who are now fully familiar with the engineering details of the CANDU reactor.

The fuel manufacturing shops in Romania have been completed and fuel is being manufactured and stored.

Commitment to the programme is evident to the highest levels in Romania, and this was obvious at the recent visit of President Nicolae Ceausescu to Canada. It included a tour of the Gentilly CANDU Nuclear Power Plant during which Mr Ceausescu entered the containment building and inspected some of the components while the reactor was at full power. He also reviewed the Romanian plans to commit further units and stated that the site for the next five reactors would be chosen before the end of this year.

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The nose knows

SIR — Once again Gaylarde has taken it upon himself to "summarize briefly" my hypothesis (*Nature* 315, 92; 1985), and once again I must urge interested readers to read the original instead (*Nature* 311, 515; 1984). It is true that "the inhabitants of the dry desert regions of North Africa and the Middle East, where natural conditions provide the most viscous air to be found on Earth, are not noted for short notes with wide nostrils", but this is not evidence of a lack of effect from climate. People in hot dry places have had to adapt to dust, because hot dry places produce dust more easily and because hot dry air supports more dust. More properly, dry particles float in dry air while humid particles (humidity gained from humid air) sink. That is why people in dusty climates have dust-trapping noses.

Regarding the irrelevance of Gaylarde's advertised "familial virtue", I only wish to be clear that I do not claim familial virtue, or even personal virtue. I claim to have made an argument which is logical and empirically reasonable. Gaylard has decided that I am either a fascist or a would-be fascist (*Nature* 313, 425; 1985 and see also *Nature* 314, 398; 1985). I would prefer to hear his thoughts about my argument, but it seems that he will never calm down enough to get it straight.

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Safety cabinets

SIR — In commenting on the relevant advantages of Class I and Class II safety cabinets (20 June, p.626), R. P. Clark deals only with the containment of aerosols. Much of my 40-year professional career as a medical microbiologist has involved work with high-risk pathogens including smallpox, lassa fever and simian B virus, and I have used all three classes of cabinets for this work. My experience leads me to say that for any pathogen in which the risk to the operator arises mainly from needle-stick type of injuries, Class II cabinets afford a significantly higher level of protection than Class I simply because of the better visibility which they afford and the better ergonomic design which is possible for Class II cabinets.

In my view it would be irresponsible to work in a Class I cabinet if a Class II cabinet was available, for this type of work.

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Why celebrate laser birthday?

Lasers were first constructed 25 years ago. An historical account and some recent work appear in this issue. There can hardly ever have been an instrument that so quickly became pervasive.

WHY celebrate the 25th anniversary of the invention of a scientific instrument, the laser? Why not instead be patient, and wait for the more conventional half-century? Or, since lasers are mostly tools rather than objects of interest in their own right, why not celebrate the discoveries their existence has made possible rather than the means by which these ends have been successfully attained? These questions are all properly provoked by the group of articles on pages 307 to 330 of this issue. What follows is the explanation.

First, the supposition that lasers are merely tools for doing an admittedly huge diversity of jobs is easily made but is also false. With all that has happened in the past 25 years, it tends to be forgotten that the development of the first working laser was also the first occasion on which it became possible to study coherent electromagnetic radiation at or near the wavelength of visible light. By contrast, the study of coherent microwave radiation was in no sense dependent on the earlier invention of the microwave analogue of the laser, called the maser.

Both devices were also, as it happens, in their time the most vivid practical demonstrations of the reality of the phenomenon, originally inferred by Einstein during the development of his quantum theory of radiation (in 1912), that an excited atom capable of emitting a photon of a certain frequency will be stimulated to do so by radiation of that frequency or something like it, and that the likelihood of stimulated emission will be proportional to the intensity of the stimulating radiation. Although there was never any reason to quibble about Einstein's conclusion, which was enthusiastically used in the 1920s by R.H. Fowler and A.S. Eddington (for example) for the calculation of the transmission of radiation through stellar atmospheres, and although the direct calculation of the Einstein coefficient became one of the first successes of the wave mechanics of 1926, it always helps to turn a phenomenon into a fact of life that there should be a direct demonstration of it.

The device constructors now have an open licence. Their objective seems simply to make every possible kind of electronic excitation into the working principle of a laser, whence the emergence of solid-state semiconductor lasers (where the excitation is that of electrons into the conduction band). The outcome is coherent radiation because the stimulated emission

is always in phase with that which stimulates it. The practical difficulties are always those of producing a sufficient supply of excited entities, electrons in atoms or in semiconductors, to swamp incoherent spontaneous emission of the same radiation. So pumping (from the ground state to the excited state) is where the designers spend their energy.

Weird and wonderful things are done in the process, as shown by an account in the current issue of *Applied Physics Letters* of an attempt by R.S. Taylor and K.E. Leopold of the National Research Council of Canada to extend the pulse-length of a xenon/chlorine excimer laser (*Appl. Phys. Lett.* 47, 81; 1985). Here the excited state is that of an ionized atom, the lower level is a loosely-bound outer electronic state and the energy difference corresponds to ultraviolet radiation. Pumping such a device entails the creation of free electrons, which means an electric discharge.

But how can such a phenomenon be sustained for a reasonable length of time, say a microsecond? The answer offered is a double bank of capacitors discharging through one half of a rudimentary transformer, one quickly (in a few tens of nanoseconds) at high voltage and the other more slowly, through the laser gas made electrically conducting by the trigger pulse. The engineering of this equipment entails arguments of the kind now most commonly encountered among those who organize the discharge of the energy of capacitors into the plasma contents of thermonuclear fusion machines (but the laser is on a much smaller scale).

Taylor and Leopold claim an efficiency of 2 per cent for their device, with a 0.4 microsecond pulse of ultraviolet radiation, and indeed look forward to the continuous operation of devices like theirs. The US Strategic Defense Initiative will be interested. Taylor and Leopold calculate that they have extracted 1.5 J of power from each litre of lasing gas in a pulse lasting only 0.4 microseconds, which corresponds to 3.75 MW at continuous operation. Pumping the materials from which X-ray lasers may be made has still some way to go.

These are the practical problems; what are the benefits? Even after so short a time as 25 years, it is easy to overlook, or to take for granted, the way in which the use of lasers has transformed whole areas of investigation. Chemists concerned with the mechanisms of chemical reactions

were among the first to appreciate the value of devices that could be used to excite molecules in specific ways and in times short compared with those of chemical transformations and interactions. One tangible outcome, for example, has been a knowledge of the role of molecules of chlorophyll in photosynthesis that was previously entirely inaccessible. Similarly, there is now a whole new field of spectroscopy, that of the highly excited atoms called Rydberg atoms, whose existence has been made possible only by the availability of tools that can be used in the preparation of the Rydberg states. The value of being able to make measurements of the properties of the species involved in, say, the upper atmosphere or in stellar atmospheres should be huge.

The most casual readers of the literature must now be aware of the pervasive influence of lasers in most fields of research. (The issue of *Applied Physics Letters* carrying the article by Taylor and Leopold has no fewer than eight other accounts of schemes for making novel lasers function, but the journal is the device-constructor's running commentary.) Recent demonstrations that lasers, by means of the momentum transferred in the absorption of photons, can be used to rob atoms of virtually all their translational velocity ("cooling" is the term) points the way to a further increase in the accuracy of atomic spectroscopy and thus to the definition of frequency standards, or time standards, more accurate than even those now used (see B.W. Petley, *News and Views* 27 June, p.716).

It is also now a matter of common knowledge that the stability of certain geological structures, critical parts of the San Andreas fault, for example, are monitored by geodetic lasers, that other instruments are used for the direct measurement from the ground of the constituents of the upper atmosphere (LIDAR) and that the accumulation of data from Earth satellites and from the corner reflectors left on the Moon during the Apollo missions will eventually yield a more precise test of current understanding of the dynamics of the Solar System, relativistic corrections included.

So there is plenty to celebrate. That lasers would turn out to have such an influence in so many different fields was foreseen 25 years ago. The surprise is that so much has happened so quickly.

John Maddox

Muscle contraction

Weak and strong crossbridges

from Malcolm Irving

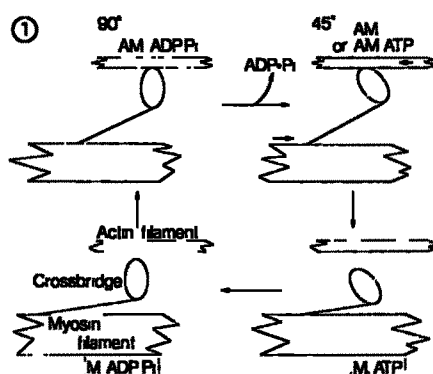
IN THE most widely accepted model of muscle contraction the actin filaments are pulled along by the cyclical action of crossbridges projecting from the myosin filaments. In this model, crossbridges are thought to attach to actin roughly at right angles to the filament axis (the so-called 90° state) and then to swing round in a power stroke that moves the actin filament by about 120 Å (refs 1,2). Binding of ATP is believed to cause detachment of the crossbridge, the ATP has to be hydrolysed before the crossbridge can rebind to actin and repeat the cycle³. This model was an attractively simple explanation of mechanochemical coupling in muscle. But it is now being suggested that crossbridge detachment is not an obligatory consequence of ATP binding⁴, that the idea of the 90° state needs major revision^{4,5} and that the power stroke is much shorter than 120 Å (ref.5). In contrast, T. Yanagida *et al.* elsewhere in this issue⁶ present an argument for a power stroke larger than 600 Å (see box). Where does this leave the crossbridge theory?

A cartoon of the original model is shown in Fig.1, where the crossbridge is drawn as a 'ball-on-a-stick'. The elongated ball is the 'head' domain of myosin (M), containing the ATP and actin binding sites. Starting at the lower left of the reaction cycle, a head with bound ADP and phosphate (P) binds to actin (A) to produce the complex AM.ADP.P_i. Release of the products of ATP hydrolysis is coupled with the power stroke as the head swings to 45° (AM). ATP binding detaches the head (M.ATP) from actin, the ATP is hydrolysed and the resulting complex, M.ADP.P_i, can rebind to actin in the 90° configuration. This biochemical scheme was suggested by studies in solution on the interaction of actin with isolated myosin heads⁷.

E. Eisenberg and T.L. Hill⁸ have recently reviewed these solution studies in the light of subsequent experiments using a wider range of conditions, it is now clear that, in solution, ATP binding to the head does not necessarily cause it to dissociate from actin before the ATP is hydrolysed. Rather, when either ATP or ADP and P_i are on the head it is weakly bound to actin, in a rapid equilibrium between attachment and detachment. A simple crossbridge cycle suggested by these newer data is shown in Fig. 2. Here there is no structural difference between the weakly bound AM.ATP and AM.ADP.P_i complexes, which have been depicted with a range of orientations to suggest the possibility that they do not have a sterically unique conformation. The power stroke is still associated with P_i release, and drives

the head into the strongly bound AM.ADP and AM states. The difference is that ATP binding now returns the head to the weak binding states rather than detaching it.

At first sight the new model seems to have a fundamental defect: the power stroke is reversed while the heads are still



attached, which must push the actin filaments the wrong way. Why does this not reverse the work done in the power stroke? Recall that a myosin filament has many crossbridges working in parallel, but asynchronously, so that swinging of an individual head will not necessarily be accompanied by movement of the actin

filament to which it is attached. The difference must be taken up as conformational strain within the attached bridge. To make the model work, Eisenberg and Hill propose that those heads in the population that are strained the 'wrong' way detach more quickly. The general idea of a strain-dependent detachment rate is not new⁹ and, indeed, is necessary for the efficient operation of any model of this general type, including that in Fig. 1. Such models can provide a quantitative description of the mechanical and energetic properties of muscle¹⁰, but are not critically dependent on the chemical identity of the intermediates in the crossbridge cycle.

Does the biochemical cycle in Fig.2, inferred from solution studies, actually occur in contracting muscle? The answer will require biochemical studies of intact muscle fibres, in which it is difficult to apply the transient kinetic techniques used in solution. Fortunately, it has recently become possible rapidly to release ATP from a photolysable precursor inside muscle fibres¹⁰, providing at least a partial solution to the technical problem.

From the structural viewpoint the model in Fig. 2 is rather poorly defined. One general problem is that if strain in a crossbridge affects its structure then a given biochemical state may not be identifiable with a unique conformation. Nevertheless, there still might be two types of conformation corresponding to the strongly and weakly bound states. H.E. Huxley and M. Kress¹¹ have now summarized the ultrastructural evidence from contracting muscle and demonstrate

A crossbridge too far ?

AN interesting new view of the crossbridge power stroke is presented by T. Yanagida, T. Arata and F. Oosawa on page 366 of this issue. They have estimated the length of the power stroke from the maximum velocity of filament sliding in crab muscle, in which the sarcomeres are long enough for the velocity of fluorescently labelled actin filaments to be measured with a light microscope. The normal transverse connection between adjacent actin filaments — the Z line — was removed by selective proteolysis, leaving single sarcomeres with a normal array of myosin filaments but with actin filaments that were now free to move independently. On addition of ATP in the presence of calcium, all the actin filaments started to slide with a velocity of about 5 $\mu\text{m s}^{-1}$. The rate of ATP hydrolysis in the same conditions was about 1 s^{-1} per myosin molecule, which corresponds to about 80 s^{-1} per actin filament.

Yanagida and his colleagues argue that as viscous drag would very quickly attenuate actin filament momentum, each filament must have at least one crossbridge pulling on it at any instant. So if one ATP molecule is hydrolysed per crossbridge cycle, the rate of cycling of active cross-

bridges could not be higher than 80 s^{-1} . To achieve a filament velocity of 5 $\mu\text{m s}^{-1}$ the length of the power stroke under these conditions would therefore have to be at least 5/80 μm or 625 Å.

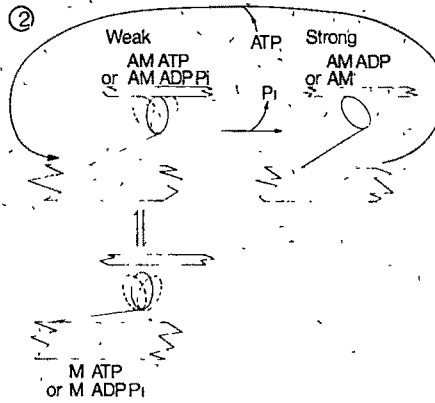
This estimate is not only strikingly larger than those obtained by other methods (see accompanying article) but also than the largest dimension of the crossbridge head (190 Å) or the actin monomer (70 Å). If the power stroke really is greater than 600 Å, some drastic rethinking of the swinging head crossbridge model would be required. The paradox might be most simply resolved, and a short power stroke retained, if the condition that one ATP molecule must be hydrolysed per cycle of crossbridge attachment and detachment did not hold during rapid filament sliding against little or no resistive force. This could occur, for example, if the number of weakly bound, rapidly attaching and detaching crossbridges increased during sliding as the degree of overlap between actin and myosin filaments became progressively greater. This might make sliding energetically favourable without committing such crossbridges to ATP hydrolysis.

Malcolm Irving

that it is consistent with the existence of two such populations.

Strongly bound crossbridges may be structurally similar to those in rigor muscle, where no ATP is present. If such heads were attached to actin in a stereospecific way and all had the same shape they would take up the periodicity of the actin filament helix. X-ray diffraction studies suggest that no more than 20 per cent of the heads are attached in this way during contraction (compared with 100 per cent in rigor). This is marginally consistent with electron spin resonance studies¹¹, which indicate that in about 20 per cent of the crossbridge heads the probed domain has the same orientation as in rigor; the rest of the heads are disordered and rapidly mobile. However, another part of the X-ray diffraction pattern shows that at least 60 per cent of the heads are near the actin filaments during contraction. Huxley and Kress suggest that in addition to the 20 per cent of strongly bound heads, a further 40 per cent are weakly bound, with a wide range of permitted orientations and in a rapid equilibrium of attachment and detachment. Independent evidence for this type of crossbridge attachment has come from studies on demembrated muscle fibres in calcium-free, low ionic strength media — conditions in which isolated heads in solution are weakly bound to actin. X-ray diffraction studies on such fibres showed that the crossbridges were near actin but had not taken up its helical periodicity¹² and, although no force is generated, mechanical studies detected a population of rapidly attaching and detaching crossbridges¹³.

Do the weakly bound crossbridges in contracting muscle also generate no force? If only the strongly bound 20 per cent of heads do so, one can calculate that each would have to exert about 6 piconewtons in a slowly shortening muscle. But if this is applied over a 120 Å power stroke the work produced per cycle (7.2×10^{20} joules) would be about three times greater than expected from ATP hydrolysis on the basis of the observed macroscopic efficiency. Huxley and Kress conclude from a similar calculation that if only 20 per cent of heads produce force the power stroke must be smaller than originally proposed — they suggest it is about 40 Å. The previously favoured value of 120 Å came from experiments in which extremely rapid shortening was imposed on active muscle²; fast force recovery was seen only if the filament sliding was less than 120 Å. Huxley and Kress suggest an alternative interpretation of these experiments in which 120 Å becomes the range of attachment of the weakly bound heads. Although these would not normally be force producing, filament sliding of up to 120 Å would bring them into the force-generating zone where they could make the transition to the strongly bound state. A 40 Å power stroke of a strongly bound head could plausibly occur in an all-or-



none fashion, storing energy in the conformational strain of another part of the crossbridge. This would explain why the ordered heads in contracting muscle have exactly the rigor conformation (as detected by spin-resonance probes): strongly bound heads are only seen at the end of their power stroke.

Many questions remain. Much of the mechanical and structural data could be explained without postulating the existence of weakly bound, non-force-generating crossbridges in contracting muscle. In an alternative view most of the heads would be strongly bound, force generating and take up a wide range of conformations with rapid transitions between them². A key proposal of the model of Huxley and Kress, that only 20 per cent of heads are strongly bound and force generating, derives from studies with extrinsic probe molecules. It will be important to check by independent methods that crossbridge properties have not been modified by the probes and that the mobil-

ity of the probe does indeed reflect that of the head. This proposal also seems difficult to reconcile with existing data from experiments designed to compare the mechanical properties of contracting with rigor muscle¹⁴ which suggest that the fraction of force-generating heads during contraction may be as high as 75 per cent. Nevertheless, the idea that there are two types of crossbridge in contracting muscle — weakly and strongly bound — provides a neat and stimulating synthesis of a wide range of muscle phenomena. In the longer term its success will depend on the extent to which these two hypothetical states stand up to attempts at making a more rigorous characterization of their structural and dynamic properties. □

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Structural geology

To stretch a continent

from I. R. Vann

THAT large areas of the Earth's crust have been subjected to compression has been known since before the discovery of plate tectonics. But, outside the world of hydrocarbon exploration, extensional tectonics has been a topic of intensive study for less than a decade. A recent conference* illustrated the remarkable progress that has arisen from two very different scientific approaches, the first a deduction straight from the world of physics, the other an induction rooted firmly in the geological tradition of careful field observation.

Seven years ago, D. McKenzie made what in retrospect seems a trivially simple prediction (*Earth. planet. Sci. Lett.* **40**, 25; 1978) — if the lithosphere is stretched then it must thin. Since the base of the lithosphere is an isotherm, a thermal anomaly is created that slowly decays causing subsidence of the crust as an inverse exponential function of time. The

power of this model is that it connects the stratigraphy and thermal history of the sediments filling a basin with a regional structure. At around the same time, field studies in the 'Basin and Range', the core of the Rocky Mountains of the western United States, began to show that the structure of the area is dominated by flat-lying extensional faults (detachments) with horizontal offsets across them of tens of kilometres.

This observation dispelled an old geological prejudice that sub-horizontal faults are all compressional structures (thrusts). Despite this a number of questions remain, concerning the original geometry of the flat detachments, the relevance of the Basin and Range model to other extensional basins and the relationship between the extension recorded near to the surface in rift basins and deeper structures.

At the conference the problems of unraveling the original geometries of flat detachments was exemplified by the con-

*'Continental Extensional Tectonics', University of Durham, UK, 18-20 April 1985

tributions of G. H. Davis (University of Arizona), and E. L. Miller (Stanford University) and her co-workers. Davis suggested that the faults and the 'metamorphic core complexes' that underlie them were originally moderately dipping ductile zones of simple shear formed at 10 km or deeper within the crust. As extension progressed, the material overlying those 'faults' was moved laterally by tens of kilometres. The overall effect of this is to bring the faults nearer the surface and thus reduce the confining pressure and temperature. This causes a change in the deformation from a ductile to an increasingly brittle process. According to Davis, younger cross-cutting extension faults later rotated the dipping zones to horizontal.

E. L. Miller's model is markedly different. She suggested that the Snake Range detachments in Nevada are neither faults nor shear zones but the transition, formed at a depth of 12 km, between an upper brittle plate and a lower ductile one, which latter deformed by pure shear expressed as horizontal extension and vertical thinning. Many other speakers accepted in modified form one or other of these apparently incompatible models but it was disconcerting for the outsider to see that this marvellously exposed area remains so enigmatic.

Even if the Basin and Range ultimately yields a single unified model it will remain a unique area of extreme extension within a region of relatively recent crustal thickening in the core of a thrust mountain belt. A possible ancient analogue — the Devonian (380 Myr old) basins of western Norway — was described by M. Seranne, M. Seguret and Ph. Laurent (University of Montpellier II). Here 50 km or more of extension occurs above a shear zone, apparently a reactivated thrust, which dips westwards at 5° to 25°, thereby creating a basin containing sediments up to 25 km thick. Geometrically similar basins, observed on deep seismic profiles (Brewer, J. A. & Smythe, D. K. *J. geol. Soc. Lond.* 141, 105; 1984), occur above similarly reactivated thrusts on the western margin of the Caledonian mountain belt in north-west Scotland, which appear to flatten as they enter the lower crust at a depth of around 18 km. These fragmentary observations suggest that an extensional province similar to the Basin and Range existed within the Caledonian mountains some 380 Myr ago.

Is it possible to carry the Basin and Range analogy further? Certainly there are great differences between the extension within mountain belts and that which creates great sedimentary basins (rifts) in old stable crust (such as the East African Rift). Perhaps the most obvious of these differences is that almost all these rifts are covered by water or thick blankets of sediment. Hence their structure is not accessible to the geologist's hammer and our understanding depends largely on seismic

reflection data that are even more ambiguous than surface observation. A. D. Gibbs (Midland Valley Exploration, Glasgow) presented models of rift fault systems developed in the North Sea that exemplify the belief of a growing number of workers that extension is achieved by movement on a linked series of faults that have flatter and steeper portions producing a 'staircase' geometry in the upper crust. These faults systems are strongly asymmetric giving rise to basins bounded on one side only by major faults. Along the length of any basin this asymmetry flips from side to side across complex transfer zones.

Unfortunately the seismic data, which has been acquired almost exclusively by oil companies, images only the upper 10 km of the crust. Basin and Range models are applicable, if at all, to rift systems at depths of more than 15 km where it is thought that the crust changes from a generally brittle to a ductile material. Evidence about deformation at these depths below rifts comes from a very few deep reflection experiments conducted mainly on the UK continental shelf. A. Beach (Britoil, Glasgow) presented one such profile across the northern North Sea. His interpretation suggests two major periods of extension in this rift with active faulting in Triassic and early Jurassic times (220–200 Myr) above a single low-angle shear zone akin to the Basin and Range model

of G. Davis being followed by extension at the end of the Jurassic along steep faults that cut entirely through the crust. The profile demonstrates that the crust below the rift is only half as thick as it is on the flanks and hence supplies one of the few direct tests of the McKenzie stretching model.

Support for Miller's interpretation of the Basin and Range came from a discussion by J. A. Jackson (Cambridge University) of deep deformation processes within active regions of extension. He noted that all major earthquakes in active regions occur on faults dipping at 30–60° at depths of 8–12 km and are grossly planar from the surface to these depths. Below this earthquakes are rare, but when they do occur (usually as aftershocks to large shallower events) they seem to do so on horizontal fault planes. Jackson concludes that extension in the lower crust normally takes place by ductile diffuse creep with only minor brittle effects.

Despite unanswered fundamental questions, and amidst much conflicting evidence and irreconcilable theory, many participants left the conference feeling that they had attended an important event. Among the conflict it seems there is a convergence that in a very few years will lead to a unifying 'general theory' of extensional tectonics. □

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Infrared astronomy

Views across the Galaxy

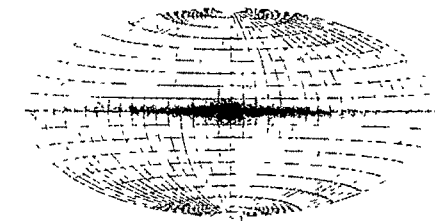
from Charles Beichman

No longer the coveted property of just a few infrared astronomers, the wealth of data returned from the Infrared Astronomical Satellite (IRAS) is now being studied by a large number of scientists working in all regions of the spectrum. Three days of a recent conference* were devoted to the presentation and discussion of IRAS discoveries within our Galaxy. More than a dozen invited talks and fifty contributed papers described what IRAS has revealed about the death and birth of stars, the large-scale structure of the Galaxy and properties of interstellar dust.

An examination of the overall statistics of the IRAS point source catalogue (T. Chester, California Institute of Technology) demonstrates the importance of IRAS to galactic astronomy. Half of the 245,839 sources detected by IRAS lie within seven degrees of the galactic plane; two thirds of the sources lie within the central two quadrants of the Galaxy. Roughly 158,000 stars within the Galaxy comprise 65 per cent of the catalogue. Some 65,000 objects are associated with different phases of the interstellar

medium, including H II regions, molecular clouds with embedded pre-main sequence stars and dense clumps of cold dust. The remaining 9 per cent of the catalogue consists of about 22,000 external galaxies. Spectroscopic data obtained with the IRAS Low Resolution Spectrometer are available for the 5,000 brightest objects (F. Oloin, Groningen).

At the end of its life, a normal star ejects a large part of its mass back into interstellar space. The resulting shells of circumstellar dust can convert most of a star's luminosity into infrared energy and make such stars detectable by IRAS across nearly the entire Galaxy. H. Habing (Leiden) used stars with shells detected at 12 and 25 microns to trace the disk and central bulge of the Galaxy with unprecedented clarity (see figure), free from the effects of inter-



*The IRAS Symposium "Light on Dark Matter" was held at Noordwijk aan Zee, the Netherlands, 10–14 June 1985.

stellar extinction that preclude such studies at visual wavelengths. The colours and luminosity of these stars resemble those of previously known OH/IR stars, which, in addition to being bright in the infrared, emit intense radiofrequency radiation in the spectral lines of the OH radical. Ground-based observations (J. Frogel, Kitt Peak; M. Feast, South African Observatory) confirm that many IRAS stars found in the bulge are late-type M giant stars, with luminosities as large as 10^3 to $10^4 L_{\odot}$, that are losing mass at a rate of 10^{-6} to 10^{-4} solar masses per year. The age of these stars and the mass of their main sequence progenitors are quite uncertain, but an intriguing possibility is that they were originally one-solar-mass stars and are no more than a few billion years old, far less than the age of the Galaxy. In that case, there may have been a burst of star formation in the bulge of the Galaxy that resembled bursts seen towards other infrared-bright galaxies detected by IRAS.

B. Zuckerman (University of California, Los Angeles) gave two reasons why an understanding of the mass loss in these late-type stars is important. First, as we observe many white dwarf stars and relatively few supernovae in the Galaxy, mass loss must provide the mechanism whereby stars more massive than 1.4 solar masses lose enough material to avoid a catastrophic demise when their nuclear fuel has been consumed. Second, it is through mass loss that the carbon, nitrogen and oxygen produced by nuclear burning are injected into the interstellar medium for subsequent processing into new stars, planets and, eventually, astronomers. By using a combination of IRAS measurements and millimetre-wavelength observations of gas outflows for a large sample of IRAS stars, it will be possible to learn much about the dynamics and chemistry of the mass-loss process and its relation to later stages of stellar evolution.

P. Bedijn (Heidelberg) explained the cause of mass loss in terms of a positive feedback mechanism coupled to global pulsations of the star. His theory derived support from the fact that more than half of the IRAS sources varied in brightness by as much as a factor of three over the course of the IRAS mission.

After the mass-loss phase ends, it is thought that late-type giants become planetary nebulae which consist of a 50,000 K star surrounded by a thin shell of ionized gas. A. Leene and S. Pottasch (Leiden) used 8 to 22-micron spectra obtained with the Low Resolution Spectrometer to show that for some of the more than 700 planetaries detected by IRAS, the radiation does not come from hot dust, but rather from spectral lines from ionized gas. Emission from spectral lines of S IV (triply ionized sulphur), Ne II, O IV and S III account for all of the radiation detected by IRAS in the 12- and 25-micron bands of the survey. Leene and

Pottasch predicted that lines of N II and O III could account for some or all of the 60- and 100-micron radiation detected in the survey for these objects. IRAS maps of planetary nebulae can be used to examine the temperature, abundance, density and excitation of these ionized species.

IRAS is also well suited to studying the other extreme of the life history of stars — their birth out of the interstellar medium. The physics of star formation represents a fundamental problem in modern astronomy which is relevant both to the structure of our own Galaxy as well as to the energy production mechanisms for the most luminous starburst galaxies detected by IRAS. As B. Elmegreen (IBM, T.J. Watson Center) pointed out, IRAS data at the highest sensitivity and spatial resolution can be used to study the detailed physics of star formation, and IRAS images can be used to study large-scale processes in the interstellar medium that produce stars.

One mechanism for the formation of stars that has been discussed for many years is that H II regions around newly formed massive O and B stars trigger the fragmentation and subsequent collapse into new stars of adjacent material in a giant molecular cloud. By examining the 60- and 100-micron images produced by IRAS, P. Schwartz (US Naval Research Laboratory) found circumstantial evidence for loops and rings of star formation surrounding earlier sites of star formation. One such loop is 250 pc in diameter and contains many H II regions and embedded young stars. If further examination of the IRAS images shows that star formation is often associated with structures of this type, then the case for triggered or sequential star formation will be greatly bolstered. As Schwartz emphasized, large-scale morphological studies of this kind are possible only with the advent of the IRAS images that present to the eye in a single picture both the distribution of the

interstellar matter and the presence of embedded young stars.

A second way by which star formation may proceed is to compress small globules of interstellar material enough to overcome the supporting motions within the cloud and so initiate a gravitational implosion that leads to the formation of one or more stars. It is possible that many solar-type stars form in this manner, rather than in the triggered fashion of the more massive stars. The existence of this more sedate and spontaneous type of star formation has been demonstrated by P. Myers (Center for Astrophysics), C. Beichman (California Institute of Technology), S. Harris and J. Emerson (Queen Mary College, London) and R. Jennings (University College, London) who examined more than 100 small clumps of interstellar gas in the Taurus and Ophiuchus molecular clouds, some 150 pc away. These clumps contain between 5 and 50 solar masses of material and represent the simplest possible sites for star formation. The IRAS survey shows that about half of these clouds are associated with infrared point sources. Although a few objects can be identified with visible pre-main sequence stars (T Tauri stars), the remainder have no visual counterparts. The invisible sources have very low colour temperatures, about 100 K, and luminosities of 0.5 – $10 L_{\odot}$. Although it is not possible to determine the masses of such objects accurately, it is likely that stars of about 0.5 – 1 solar mass are being formed in these clumps.

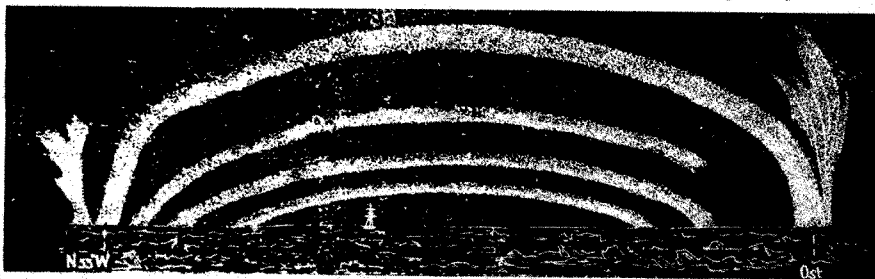
The most important, but as yet unanswered, question about these embedded infrared sources concerns their evolutionary status. Some of the invisible infrared objects are probably T Tauri-like stars, no older than 10^5 years, that obtain their energy from the slow contraction of a stellar core in quasi-static equilibrium, but which are still obscured from view by placental gas and dust. On statistical

100 years ago

MR TROMHOLT has rendered a great service to science by the travels and observations recorded in these volumes¹; indeed, it would not perhaps be going too far to say that we have here, brought before us in the most interesting manner, one of the best organised attempts to study the aurora that has been made for many years, the credit for which must be given to the organisers of the International Polar Research Expedition of 1882–83. The results, however, recorded in these volumes are by no means limited to the height of the aurora. The con-

THE AURORA

stant study afforded to Mr. Tromholt of one of the most awe-inspiring phenomena which it is given to man to witness have permitted generalisations to be reached and hypotheses to be broached of the greatest scientific interest. Mr Tromholt holds that *the auroral zone moves northwards and southwards daily, yearly, and eleven yearly*. To speak in vacuum-tube language, instead of one rigid stria, we may have many striæ, and these moving towards or away from the auroral pole as ordinary striæ move towards or away from the negative pole.



¹Under the Rays of the Aurora Borealis. By S. Tromholt. Edited by Carl Siewers. (London: Sampson Low & Co., 1885.)

From *Nature* 37 274, 23 July 1885.

grounds, however, it is probable that some of these IRAS sources are still deriving most of their luminosity from the release of the gravitational energy of infalling cloud material. These objects can be considered to be true protostars. Confirmation of this status and further study must rely on observations at other wavelengths, in particular at high spatial and spectral resolution in the near-infrared and at millimetre wavelengths.

Infrared astronomy can be considered to be the study of dust in one form or another, and a large part of the conference reflected the importance of solid material, heated enough to be detectable in the infrared. The so-called infrared cirrus, diffuse emission from interstellar dust, is an important and puzzling discovery of IRAS. T. N. Gautier (California Institute of Technology) and others showed that at 100 microns the cirrus can be correlated with faint dust features visible on high-contrast optical photographs and with structures seen in 21-cm maps of atomic hydrogen (H I). Some cirrus can be associated with weak emission seen in high galactic molecular clouds.

Some of the most exciting results concern the diffuse emission seen at 12 microns by IRAS. The properties of 12-micron cirrus tend to confirm the work of K. Sellgren (Hawaii) that there exists dust whose apparent colour temperature is much hotter than its equilibrium temperature. The explanation of this effect is that motes of dust a few tens of angstroms in size can be heated, briefly, by the absorption of a single ultraviolet photon. The heated grains cool off by emitting radiation at anomalously hot temperatures. A. Leger, J. L. Puget and F. Boulanger (Paris) have identified polycyclic aromatic hydrocarbons as a likely type of grain material. Composed of carbon and hyd-

rogen ions, they are not only stable at the small sizes required, but also have spectral features at 3 and 11 microns that match previously unidentified bands found towards several infrared sources.

The realization that very small grains play an important role in the interstellar medium was brought out repeatedly during the conference. Elmegreen pointed out that if very small grains exist at the centres of dense molecular clouds and not just at their periphery, then they will be charged and will control the coupling of the gas to the magnetic field. The properties and abundance of these grains may control the collapse of molecular clouds.

The 60- and 100-micron IRAS maps have been used to examine the large-scale structure of the interstellar matter in the Galaxy. B. Burton, H. Walker and E. Deul (Leiden) found that the scale height of the 100-micron emission is about 120 pc, the same as that of the atomic hydrogen (H I), and twice as great as the scale height of the molecular gas as inferred from CO observations. Thus, IRAS has traced the distribution of warm (22 K) dust in relatively diffuse regions and not the distribution of the colder, denser material. A longer wavelength map of the Galaxy, say at 200–300 microns, would trace the 10–15 K material associated with the molecular component of the Galaxy.

The IRAS catalogue and all sky images have been generally available only since last December. The number and variety of results reported at the conference suggest that the data returned by IRAS will be a valuable resource for astronomers for years to come. □

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Infrared astronomy

Views outside the Galaxy

from Martin Ward

ALTHOUGH the infrared satellite mission IRAS ended its life more than a year and half ago, its influence on the subject of infrared astronomy promises to continue for many years to come, by virtue of the enormous database of observations that it produced. That this is so for extragalactic astronomy was demonstrated at a recent workshop*, the main thrust of which was aimed at the understanding of aspects of star formation within galaxies.

Young stars are hot and emit most of their energy in the optical and ultraviolet regions of the spectrum. But as dust and gas exist together in the star-forming regions, some of these optical and ultraviolet photons heat up the dust grains,

which then radiate approximately like black bodies. The exact temperature of the heated dust will depend on various factors but, leaving aside the more exotic active galactic nuclei for the moment, the range extends from 30 to 100 K. These temperatures produce peak emission in the infrared, hence the connection between star formation and far-infrared emission.

As was emphasized by H. Yorke (University of Göttingen), there are still fundamental questions that need to be answered concerning star formation and the evolution of H II regions. For example, relatively how many new stars of various masses are formed in any one episode of star formation? Since high mass stars evolve faster than those of low mass,

the differences in relative numbers will manifest themselves as changes with time in the integrated properties of the burst of star formation ('starburst').

There are several ways in which the efficiency of star formation may be related to large scale morphological properties of galaxies—another fundamental problem. For example, T. Harwarden (Royal Observatory, Edinburgh) suggested that the presence of galactic bars may provide a kinematic catalyst which channels material inward to sites of galactic nuclear starbursts. An obstacle to progress in this area is the sheer difficulty of quantifying objectively the pictorial evidence of bar structure. Another process that may enhance the efficiency of, and/or provide a trigger mechanism for, a burst of star formation is that of galaxy interactions. R. Joseph and co-workers (Imperial College, London) have surveyed interacting pairs of galaxies and find evidence for starbursts to be very common, although characteristically only in one member of a pair.

The key observational inputs to star formation models were discussed by C. Telesco (NASA Marshall Space Flight Center). An important parameter is the total infrared luminosity of a star-forming region which, roughly speaking, indicates the number of young stars present. For this purpose the IRAS observations are ideal, covering as they do a range of 12 to 100 microns. Another global property is the mass/light ratio. In the conventional units normalized to the solar values it is known that an old stellar population has a ratio of greater than three, whereas it is predicted that the ratio for an unevolved stellar population (formed with the mass distribution observed in the solar neighbourhood) is less than 0.01. This index can therefore be used as a measure of the age of the stellar population and hence of the presence and strength of a starburst. Telesco described the application of a starburst model to the famous galaxy Messier 82. The implied age of the starburst in M82 turns out to be 20 million years. Using the far infrared luminosity, the deduced rate of star formation is 34 solar masses per year which, integrated over the age of the starburst, is a large fraction of the total mass. To avoid the situation in which such a large fraction of all mass has been converted, it is necessary to impose a cut-off in the mass distribution of the new stars. Thus, in luminous starbursts there would be relatively few new low-mass stars, high-mass conversion rates and short-burst durations. These findings will have important implications for models which follow the evolution of starbursts.

The blue compact galaxies, also known as extragalactic H II regions, are relatively unevolved stellar systems. Their near-infrared colours indicate that they are young and/or metal-deficient, meaning that relatively little mass has been converted into heavier elements via stellar evolution. In support of this, IRAS obser-

* The meeting on Extragalactic Infrared Astronomy was held at the Rutherford Appleton Laboratory, 20–27 May 1985.

variations show that the gas/dust ratio in such galaxies is a thousand times higher than that in the solar neighbourhood (P. Gondhalekar, Rutherford Appleton Laboratory); the most probable implication is that there is a pronounced paucity of dust. If dust is formed in stellar atmospheres, the low dust content implies either a slow rate or late start of star formation. It is still controversial as to whether we are witnessing the initial starburst or whether these galaxies have experienced a series of previous bursts.

Naturally the more exotic examples of active galaxies also received some attention. I reported new infrared results for an X-ray selected sample of Seyfert galaxies. Although there are exceptions, in most cases the dominant mechanism responsible for the strong infrared emission is not a massive burst of star formation such as observed in M82. It seems rather that the near infrared emission at a few microns is related, but not necessarily via the same physical process, to emission from the very compact regions. Good correlations are found with the strengths of the very broad emission lines and the X-ray emission, whilst at 20 microns the best correlation is with the narrow emission lines which originate in a volume of typically a thousand light years. This 20-micron luminosity could come from warm dust (200 K) mixed in with the line-emitting gas and heated by the very compact source at the centre of the active nucleus.

Because of the large apertures of the IRAS observations, their relation to the compact active nuclei in Seyfert galaxies is not clear. It seems probable that we are seeing a 'mixture' of several spatially-distinct components. Michael Rowan-Robinson (Queen Mary College, London) showed how, for Seyfert galaxies, the IRAS data can be separated into three

components: a cooler disc component (seen in more normal galaxies), a warmer starburst component and a Seyfert component peaking at 25 microns. The latter could be radiation from a mini-quasar at the centre of the Seyfert absorbed by dust and re-radiated in the infrared.

The cosmological implications of the IRAS observations were also considered by Rowan-Robinson. Concentrating on the galactic polar caps, where confusing effects of the infrared 'cirrus' (see *Nature News and Views* 308, 224; 1984) are smallest, he showed that there is a significant anisotropy in the number density of IRAS 60-micron sources between the north and south polar caps.

This seems not to be a local phenomenon since an identification programme carried out for the northern cap reveals that, after elimination of obvious stars, virtually every source is a galaxy and that the typical distance of IRAS galaxies is about 200 megaparsecs. Assuming that the infrared radiation traces the matter, it is possible to map the gravitational field and determine its dipole component. The direction of this component is in good agreement with the observed anisotropy in the intensity of the cosmic microwave background radiation. Thus this anisotropy could be explained as the result of the net gravitational pull on our local group of galaxies by the distribution of galaxies within about 200 megaparsecs.

Rowan-Robinson also showed that the 100-micron background radiation detected by IRAS can be separated into components due to interplanetary dust, interstellar dust and a roughly isotropic component which may be of cosmological origin. □

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Solid-state chemistry

Topotaxy in metal-oxide reduction

from Robert W. Cahn

A THIN metal film deposited by evaporation or sputtering on, say, a cleavage surface of sodium chloride is apt to be oriented in a reproducible way with respect to the substrate — this is the commonly found physical process of epitaxy, where the substrate is inert and unaffected. The chemical analogue is a topotactic reaction¹: here a parent phase undergoes a reaction and the product phase has one or more defined orientations relative to the parent. Such reactions have been analysed by metallurgists (although they never use the term topotaxy) ever since the discovery of X-ray diffraction: the key investigations on so-called Widmanstätten reactions in metallic solid solutions were carried out in the early 1930s. Metallurgists usually analyse

such reactions in terms of the fit of parent and product phases at their mutual interface.

The reverse reaction, reduction of an oxide to metal, has occasionally been examined from a kinetic point of view, especially in connection with the industrially important reduction of wuestite (FeO) to iron², but the first study of topochemical aspects has only just been performed. The results, reported by A. Revcolevschi and G. Dhalenne on page 335 of this issue³, open up a new field of research.

In chemistry proper, topotaxy is usually demonstrated in dehydration reactions — for example, the conversion of Mg(OH)₂ into MgO — or the conversion of one oxide of iron into another; other instances include breakdown of complex silicates

into simpler ones, with release of water⁴. In all these reactions, the observed orientations imply that the network of oxygen ions remains more or less intact throughout the course of the chemical reaction.

The most studied category of topotactic reaction has been the oxidation of metals. The nature of both the topotaxy of a thin oxide layer and the oxidation kinetics depends not only on the oxidation conditions but also on the orientation of the metal surface (see refs 5 and 6 for review).

Revcolevschi and his collaborators at the University of Paris-Sud have for some time studied the orientation relationships between the phases of lamellar eutectic structures consisting of two metallic oxides (complementing the substantial body of information on such relationships as regards metallic eutectics⁵). For their new experiments, Revcolevschi and Dhalenne³ have directionally solidified a eutectic of NiO and calcia-stabilized cubic ZrO₂ producing what is essentially an interpenetrating pair of epitaxially related single crystals which they then treat with a CO/CO₂ mixture that reduces the NiO to nickel without affecting the ZrO₂. The nickel proved to be in parallel orientation to the NiO which preceded it; the ZrO₂ phase acted as crystallographic reference. Just as earlier topotactic experiments indicated that the oxygen skeleton was maintained and unchanged, so the new experiment³ indicates that the nickel-ion skeleton remains fixed.

This pioneering experiment opens the way to various topotactic studies. One might try to mimic Gwathmey's celebrated experiment on the oxidation of a spherical copper monocrystal (showing the sharp anisotropy of oxidation rates and topotaxy) by reducing an oxide sphere, or to study the orientation dependence of reduction kinetics. It is known that the reduction rate of wuestite is sensitive to the population of point defects — it would be interesting to see whether orientation relationships are affected by defects.

Revcolevschi and Dhalenne's observations have a potential practical interest: by reducing NiO without altering ZrO₂, it is possible to make a lamellar product where adjacent lamellae are electronically and ionically conducting. Such a structure can perhaps be applied in photoelectrolysis and novel solid-state batteries. □

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Plant ecology

Fertile arguments in the desert

from Jonathan Silvertown

ECOLOGISTS have been arguing recently about whether interspecific competition is responsible for observed patterns of animal distribution and abundance^{1,2}. Whereas interspecific competition once seemed to be the only process of interest to the theoretically minded, now dispersal, predation, mutualism and biogeographic history are also being considered (see ref. 1). Strangely, the recent debate has occurred almost exclusively among animal ecologists but similar arguments among plant ecologists now seem to be approaching the same conclusion.

Plant ecologists studying species distri-



Larrea bushes in the Chihuahua desert.

bution in deserts have investigated the cause of the regular spatial patterns of distribution often found in the southwestern deserts of North America and elsewhere. The commonest shrub in the Chihuahua, Sonora and Mojave deserts is the creosote bush *Larrea tridentata*, which is a conspicuous part of the sparse vegetation. Bushes, or clumps of bushes, commonly have large areas of bare ground between them and often appear regularly spaced. F. W. Went suggested that this regular spacing is the result of allelopathic (toxic) interactions between bushes³. Since then, in one of the first quantitative analyses of the dispersion pattern of *Larrea*, S. R. J. Woodell *et al.*⁴ showed that *Larrea* bushes are indeed regularly spaced at sites of low rainfall but are aggregated at sites of high rainfall. In an alternative hypothesis they suggested that the regularity is the result of competition for water between roots of adjacent bushes, with reduced competition at sites of high rainfall allowing aggregation.

M. G. Barbour^{5,6} found that regular patterns of *Larrea* were in fact quite rare. D. J. Anderson⁷ further questioned Woodell *et al.*'s claim that plants in lower rainfall areas compete for water, because bushes at these sites are at such low density that each plant actually has more available soil water than in high rainfall areas where bushes are more crowded. T. J. King and Woodell⁸ replied that the quantity of water available to bushes cannot be calculated simply from density and rainfall measurements because plant size,

surface-water runoff and infiltration of water into the ground also has to be considered. They also suggested that periods of severe drought in the past might at some sites be responsible for present-day distributions. Thus, in defending the competition hypothesis, they acknowledge the importance of other factors in structuring *Larrea* communities, as animal ecologists have had to do in defence of similar arguments about interspecific patterns⁹.

The parallel with the debate among animal ecologists can be taken further. Reassessment of competition in animal ecology has been stimulated by the use of 'null models'¹² that predict how community structure would appear in the absence of competitive interactions between species. T. A. Ebert and G. S. McMaster¹⁰ have used this approach to challenge the regularity of spacing patterns in *Larrea*, which they believe may be no more than an artefact of sampling methods, by which plants that grow very near one another are recorded as a single individual. They show that this can cause a random pattern to appear erroneously as a regular one. King and Woodell¹¹ dispute this claim, but do not explain how the competition hypothesis accounts for regular spaced clumps.

A general conclusion from the use of null models in animal ecology has been that patterns can only provide correlative evidence of the processes that structure communities. Experiments are needed to test hypotheses generated by analysis of pattern distribution. Several relevant experiments have now been performed with desert shrubs. In the field, removing neighbours of individuals of both *Larrea*

and another desert shrub, *Ambrosia dumosa*, affects the physiological water status of other plants^{12,13}. Even though this is evidence of competition for water, some of the experimental populations were aggregated. Clearly, competition does not lead inevitably to regular spacing.

We can say more than this. The desert perennial *Eriogonum inflatum* with competitors nearby is more likely to die than those plants with more distant neighbours, yet this does not change the degree of aggregation in the population. The most elaborate recent experiment¹⁵ shows how uncertain the role of root competition in producing regular spacing has become. Despite a fall in shrub density, W. H. Schlesinger and C. S. Jones found no change in the dispersion pattern of *Larrea* and *Ambrosia* at a Mojave site that has been deprived of surface water runoff by a drainage system for 45 years.

Ironically, the unknown causes of regular spacing in desert plant communities may bear some relationship to the way in which animal and plant ecologists have ignored each other. Few have considered, for example, that these plant patterns could result from the activities of seed-gathering rodents¹⁶ which forage and remove seeds between bushes and which are ubiquitous in these deserts. □

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Excitation-contraction coupling

The messenger across the gap

from Andrew P. Somlyo

EXCITATION of the surface membrane of muscle results in release of calcium that is stored in an intracellular membrane system, the sarcoplasmic reticulum (SR); the Ca^{2+} released into the cytoplasm, acting through regulatory sites on Ca-binding proteins, initiates contraction. The signal from the membrane is communicated to the SR at specialized regions (triads, diads or surface couplings) where the cytoplasmic leaflets of the SR are connected to the surface membranes by quasi-periodic bridging structures across a 12–20 nm gap (refs 1,2; see figure). A major unresolved problem is the mechanism by which depolarization (or some other change in the surface membrane) is communicated

across this gap, to increase Ca^{2+} release from the SR lumen into the cytoplasm. Hypotheses to answer this question have been proposed and some, like Peter Pan, retain their youth with sustenance from the Never Land. Will the suggestion that inositol triphosphate is the messenger across the gap, as put forward for skeletal muscle on page 347 of this issue³, stay forever young?

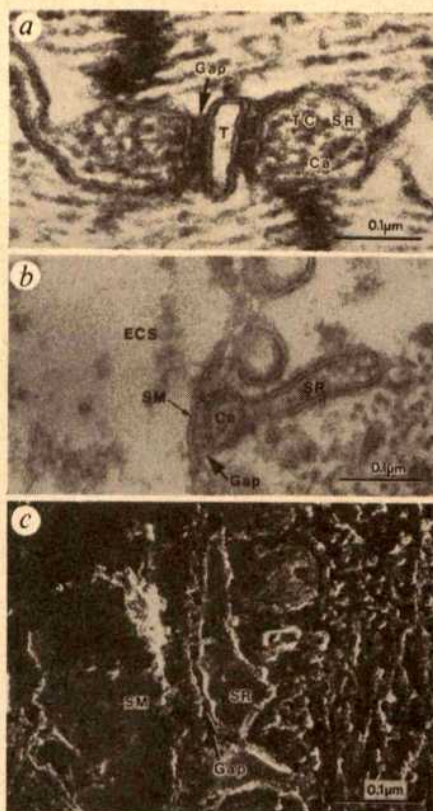
Three main classes of mechanisms have been suggested for excitation-contraction (EC) coupling: first, direct spread of ionic current from the extracellular space through the bridging structures into the SR⁴; second, mechanical transduction via a mobile protein between the two mem-

brane systems, initiated by voltage-dependent movement of fixed electric charges in the surface membrane³; and finally, chemical transmission by a diffusible second messenger. The absence of an ionic current between the extracellular space and the SR together with the fact that Ca^{2+} release is not electrically silent⁶ have excluded the first mechanism^{4,6}, and no experimental test of the second hypothesis has yet been performed. Meanwhile, as the idea of EC coupling by chemical transmission is becoming increasingly attractive, much recent evidence suggests that inositol 1,4,5-trisphosphate (InsP_3) is the second messenger⁷ in many cells. In non-muscle cells, InsP_3 is released by the transmitter-initiated hydrolysis of polyphosphatidyl inositol in the surface membrane and it is thought to mediate transmitter-induced calcium release from the endoplasmic reticulum, a structure that is homologous with SR in muscle. The inevitable question is whether InsP_3 is also the messenger in EC coupling.

When considering EC coupling, it is important to remember that there is an additional mechanism involved in smooth muscle. Whereas in both smooth muscle and striated (skeletal and cardiac) muscle contraction is triggered by electrical depolarization of the surface membrane and its invaginations (the T-tubules) and usually by an action potential, in smooth muscle, neurotransmitters also stimulate contraction or cause relaxation through a 'pharmacomechanical coupling' independent of changes in membrane potential.

Until the discovery of InsP_3 , the most popular candidate as the messenger in EC coupling was Ca^{2+} itself. According to this theory the rapid influx of small quantities of calcium current carried by the action potential triggers the 'calcium-induced calcium release' from the SR, which provides the larger quantity of Ca^{2+} required for initiating contraction. The strongest evidence for calcium-induced calcium release⁸ is in cardiac muscle, much of it from the elegant studies of Fabiato⁹. In skeletal and smooth muscle, however, Ca^{2+} can be released from the SR even in the absence of extracellular Ca^{2+} (and, therefore, Ca^{2+} -influx); nevertheless it is still possible to argue that trigger calcium is released from a protected region, perhaps the cytoplasmic leaflet of the plasma membrane^{4,10}.

The new experiments³ show that in rabbit skeletal muscle, InsP_3 satisfies the first requirement of an EC coupling messenger — it can release Ca^{2+} from the SR. This is demonstrated both with measurements on isolated SR vesicles and by the contractile response of skinned (membrane-free) muscle fibres to InsP_3 . However, such contractions are small, slow and somewhat variable, and seem to require abnormally low free Mg^{2+} concentrations to prevent the rapid breakdown of InsP_3 . None of these objections, as Volpe *et al.* and



Electron micrographs of a triad in striated muscle (a) and surface coupling in smooth muscle (b, c); c has been freeze-etched and rotary shadowed to give a clear view of the junctional gap between the sarcoplasmic reticulum and surface membranes. T, T-tubule, an invagination of the extracellular space in striated muscle; TC SR, terminal cisterna of the sarcoplasmic reticulum; SM, surface membrane of smooth muscle (a from ref. 2; b, c, from ref. 19).

others taking a similar approach¹¹ would argue, are insurmountable. For example, in live muscle, InsP_3 may be released into the diffusionally-restricted junctional space between the SR and surface membranes (see figure); this could cause a rapid and large rise in the local concentration of InsP_3 near the terminal cisternae of the SR where Ca^{2+} is thought to be released. Such regions could even contain lower concentrations of free Mg^{2+} and of the (Mg^{2+} -dependent) phosphatases that break down InsP_3 . If the electron-lucent core of the bridging structures is a lipid, as has been suggested², it could contain InsP_3 precursors. But, for now at least, these are *ad hoc* explanations of the failures of experiments to support the hypothesis.

A more devastating blow to the role of InsP_3 as a physiological messenger of electromechanical coupling in skeletal muscle would be if depolarization were found to increase only the turnover of (mono)phosphatidyl inositol, and not that of the polyphosphatidyl inositol precursor of InsP_3 , and/or if an increase in InsP_3 were shown to be a slow side-effect of acetylcholine released by nerves, and did not precede twitch contraction. The increased phosphatidyl inositol turnover evoked in frog skeletal muscle by depolarization with potassium is far more prolonged than

the duration of Ca^{2+} -release¹², and InsP_3 seems to cause only very modest calcium release from isolated cardiac SR¹³.

The most compelling evidence for a physiological role of InsP_3 in muscle is neither in electromechanical coupling nor in striated muscle, but as a transmitter of pharmacomechanical coupling in smooth muscle. In these tissues various physiological transmitters (cholinergic, adrenergic and peptides) can stimulate the breakdown of polyphosphatidyl inositol to produce InsP_3 (refs 14–16). It is important to note that depolarization does not stimulate breakdown of polyphosphatidyl inositol in smooth muscle^{14,15}, whereas cholinergic agents can stimulate such breakdown during pharmacomechanical coupling¹⁵. The release of calcium from smooth muscle cells by InsP_3 has been demonstrated in two laboratories using different methods^{17,18}. Furthermore, low micromolar concentrations of InsP_3 can cause sustained, graded contractions of smooth muscle even in the presence of the highest free Mg^{2+} (1–2 mM) likely to be present *in vivo*¹⁸. Therefore, it is probable that InsP_3 mediates at least the part of pharmacomechanical coupling that is caused by a voltage-independent release of Ca^{2+} from the SR, and seems to be similar to Ca^{2+} release from the endoplasmic reticulum of non-muscle cells.

It remains to be determined whether the effects of InsP_3 on the SR of skeletal muscle will turn out to be a major physiological mechanism or simply an interesting laboratory phenomenon like the effects of fatty acids and adenine nucleotides, which can release Ca^{2+} or potentiate Ca^{2+} -induced Ca^{2+} -release. The new findings will, at the least, stimulate the hunt for an identifiable messenger of EC coupling. In the background, like the crocodile following Captain Hook, the spectre of a more physiologically active inositol phosphate lurking in InsP_3 preparations haunts investigators in this exploding field. □

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Laser physics

Advances in free-electron lasers

from M.W. Poole

FREE-electron laser (FEL) research is continuing to break new ground; experimental configurations already exist and further modifications are being planned. The potential rewards for the successful development of such a coherent radiation source are very great, whether for radar and telecommunications, fusion energy research, photochemical processing (such as isotope separation) or fundamental research in topics such as non-linear spectroscopy. Ambitious plans are being laid for a 500-Å device and this technology is probably at least as promising as any other for the ultimate goal of an X-ray laser.

The recent announcement that yet another group has joined the increasing band of successful FEL experimenters has significance beyond its initial results. The massive resources of the Lawrence Livermore National Laboratory in California, aided by scientists from Berkeley, have now been brought to bear on the development of this exciting new technology. It seems inevitable that they and their major rivals at the Los Alamos National Laboratory could dominate future progress. Their latest results have demonstrated both exceptionally high gain ($\sim 3,000$) and attractive energy-transfer efficiency (~ 5 per cent) for millimetre waves and the race is now on to extend this to shorter wavelengths.

So far there have been two very distinct FEL communities: those using electron accelerators with beam-current densities sufficiently low that energy transfer is dominated by a single-particle (Compton) interaction, and those with access to intense beams where collective (or Raman) effects become important. The former groups have led the way since the first FEL demonstration by John Madey's team at Stanford University in 1976; important developments were summarized last year in these columns (Pidgeon, C.R. *Nature News and Views* 308, 772; 1984) which noted that four oscillators had operated over the range 0.6–10.6 μm , and since then a fifth device has extended this to 400 μm at the University of California, Santa Barbara. In fact the intense-beam workers could claim a better pedigree that extends back to the ubitron — an early centimetre wave device — and the cyclotron maser that was the centre of attention at the Naval Research Laboratory (NRL) in Washington at the time of the Madey experiments. It was the chance detection of submillimetre output from a cyclotron maser, caused by interaction of the electron beam with its 20-mm generated radiation reflected off a window, that first initiated a long programme of FEL work at

NRL over the past decade, culminating in the reporting of huge gains (120 dB m^{-1}) at 35 GHz (Gold, S.H. *et al. Phys. Rev. Lett.* 52, 1218; 1984). However, all intense-beam FEL experiments until recently have found it necessary to use an additional solenoidal field to focus the large electron beams along the length of the interaction region; such a field can contribute strong cyclotron emission that has led to ambiguities in the interpretation of the output from these devices.

What the Livermore/Berkeley collaboration has been able to announce (Orzechowski, T.J. *et al. Phys. Rev. Lett.* 54, 889; 1985) is a 35 GHz amplifier with high gain and efficiency that exhibits remarkably good agreement with theoretical expectations over a very wide range of signal levels. The experiment was started with the aim of extending collective FEL outputs down to wavelengths similar to those of the Compton devices; this would, for example, allow immediate application of the FEL to plasma heating of fusion mirror machines. Since then, President Reagan's Strategic Defence Initiative has injected financial adrenalin into FEL activities and high power at visible wavelengths is now the declared objective.

The new amplifier, ELF (electron laser facility), is based on the Livermore experimental test accelerator, a linear induction device that can deliver beams of up to 10 kA at 4.5 MeV, although only for short pulses of 30 ns at about 1 Hz. In fact the experiments have been carried out at slightly lower energy and with the electron current heavily collimated to about 500 Å to reduce the beam cross-section in the interaction region. A 3-m-long pulsed electromagnet of 98-mm periodicity induces the required energy transfer between the electrons and an accompanying wave supported by a thin stainless steel waveguide. Additional quadrupoles assist the electron beam focusing but there is no axial field.

First, the team explored the 'super-radiant mode' (laser terminology for amplification from an initial noise signal, in this case the spontaneous synchrotron radiation emitted by a relativistic electron in any magnetic field) and achieved an amplification of 13.4 dB m^{-1} down the periodic magnet. Next, a 60-kW pulse from a magnetron was injected into the interaction region and emerged as a very impressive 80 MW peak output power. In fact the FEL goes into saturation after about 2.2 m and thereafter its power oscillates along the length of the interaction.

A crucial feature of the Livermore approach has been to develop theoretical models in parallel with the experiment-

ation; this has involved extensive computer simulations to include such three-dimensional behaviour as the off-axis electron trajectories and the transverse electromagnetic mode characteristics. Their reward has been an encouraging agreement of predicted and observed FEL behaviour that sets a standard for others to emulate. In the near future the group intends to investigate enhanced efficiency to push the saturated power to still higher levels. At present the efficiency limit is set by the loss of energy from the electron beam detuning the resonant interaction between the charged particles and the wave. To overcome this the Livermore periodic magnet has 15 separate power supplies that can progressively lower the field strength along the interaction region to match the reducing electron energy, thus maintaining the resonance condition. Eventually, Livermore hopes to switch the experiment to an even more powerful induction linac, the advanced test accelerator; this might then permit the same extraordinary performance to be achieved in the infrared region.

Meanwhile, the single-particle FEL community has not been idle. A revolutionary new oscillator in the far infrared has been produced by Elias, one of Madey's original team at Santa Barbara. This takes a giant step towards extremely high average power FEL operation, because the accelerator is a 3 MV Van de Graaff facility that can in principle provide continuous wave electron beams rather than the low-duty cycles typical of most other accelerators. To achieve this would require almost all of the electron beam to be recovered after passing through the FEL, returning it to the high-voltage terminal by a suitable deceleration and collection process; this group has already demonstrated that this can be done. A laser with multikilowatt average power output cannot be far away.

The United Kingdom Heriot Watt-Glasgow-Daresbury collaborative FEL project is now fully assembled at the Kelvin Laboratory, East Kilbride. Spontaneous emission has been detected at both infrared and visible wavelengths, the latter making use of higher harmonic emission generated in the powerful periodic magnet system. Laser oscillation above threshold at 10.6 μm is now being attempted and it is also hoped to demonstrate a wide tuning range. Subsequently to this it is intended to study FEL action on a higher harmonic, a topic of great current interest as it promises to extend FEL operation down to wavelengths previously considered unattainable on such medium-energy (10–100 MeV) accelerators. Apart from this project, sadly almost all funded FEL research is now concentrated in the United States. □

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Pesticides and the decline of Guam's native birds

SIR—Diamond¹ recently reviewed the factors which might be responsible for the decline of Guam's native birds. Although Diamond's commentary was based on a report by Jenkins² that included several hypotheses (pesticides, introduced predators and disease), Diamond suggested that pesticides were the most likely cause.

The pesticide hypothesis evolved from two reports. In 1946, Baker³ stated the organochlorine pesticide DDT was used extensively on the island during and after the Second World War. In 1977, Draho⁴ reported concentrations of DDE, a metabolite of DDT, of less than 0.4 p.p.m. in the carcass lipid (about 0.03 p.p.m. whole carcass wet weight) or swiftlets (*Aerodramus vanikorensis bartschi*) and concentrations of DDT and DDE of less than 0.10 p.p.m. (believed to be on a dry weight basis) in swiftlet guano.

Diamond¹ and others^{2,4} considered these data indicative of potential harmful effects of DDT, a conclusion not supported by critical evaluation of Draho's data or concentrations of DDT and DDE (≤ 0.2 p.p.m. dry weight) detected in swiftlet guano I collected in 1981. The carcass residues reported by Draho⁴ indicate that swiftlets were exposed to DDT or DDE, but concentrations are a fraction of those associated with mortality (> 600 p.p.m. in carcass lipid)⁵ or reproductive failure (78 p.p.m. whole carcass wet weight)⁶ in birds. Residues in the guano are difficult to interpret because relationships between concentrations of organochlorines in bird faeces and those in their carcasses have not been determined. These relationships have been determined for bats, however, and residues in swiftlet guano are similar to those in guano of bats (*Myotis grisescens*) whose carcasses contained only a fraction of lethal levels⁷.

Soil and avian prey I collected in 1981 were essentially free of organochlorine pesticides; only a sample of skinks (*Carlia fusca*) contained a detectable concentration (DDE at 0.04 p.p.m. wet weight, 0.13 p.p.m. dry weight). Residues in shrews (*Suncus murinus*) trapped in 1981 did not exceed 0.30 p.p.m. wet weight. These levels are also less than those known to adversely affect birds or small animals.

In addition, it is unlikely that present use of pesticides by civilians or the military on Guam is responsible for the decline. Agricultural lands comprise less than 1% of the island's total area and more than 84% of the pesticide used is organophosphates or carbamates⁸, chemicals that do not persist in the environment⁹. Of the four pesticides used the most (malathion $>$ diazinon $>$ naled $>$ carbaryl), only diazinon has been implicated in wildlife mortality elsewhere⁹. Malathion is also the pesticide used in the greatest quantity by the military, and with few exceptions,

use has been restricted to housing areas. Although spray operations by the military may have temporarily decreased insect abundance, such decreases cannot explain the continuing decline of the island's avifauna.

It is unfortunate that Diamond¹ and others^{2,4} incorrectly equated pesticide use or residues on the island with harmful effects. Although the use of some pesticides can be hazardous to birds, and effects associated with the use of DDT still persist today^{9,10}, there are no data supporting the hypothesis that pesticides are responsible for the continuing decline of Guam's native avifauna.

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SIR—In his *News and Views* piece on the "Possible effect of unrestricted pesticide use on tropical birds"¹, Diamond used Guam's avifauna as an example based on Jenkins' report *The Native Forest Birds of Guam*². Jenkins suggested that pesticide usage may be responsible for the drastic decline of Guam's birds. However, due to publication time lag, Jenkins' monograph is only current through 1979. Research since that time suggests that although pesticides were extensively used on Guam, they do not appear to be responsible for the general decline of the avifauna (C. Grue, personal communication). However, pesticides may have impacted certain species in the past, such as the insectivorous Vanikoro swiftlet and sheath-tailed bat.

My research has focused on the possible roles of introduced avian diseases and predators. Between 1982 and 1984, a variety of birds were sampled in collaboration with the USFWS National Wildlife Health Laboratory for parasites, bacteria and viruses and two sentinel experiments were conducted. To date, no infectious organisms have been isolated that could account for the current extinctions and range reductions.

Feral dogs, cats and rats are a problem on all major islands in the Marianas. The only predator unique to Guam is the brown tree snake, *Boiga irregularis*. The snake is the major cause of the decline of

the forest avifauna. This species was introduced to Guam in 1946, and its range expansion corresponds to the avian range contraction. Predation intensity on bird-baited traps is extremely high, and alternative prey items have also declined. Supporting data are currently being prepared for publication.

Thus, although Guam may not be the best example for the dangers of unrestricted pesticide usage, it certainly should serve as a warning of the potential disastrous effects of introducing exotics to island ecosystems.

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Electrostatics revindicated . . . classically . . .

SIR—Berezin¹ has considered the minimum energy configuration of N point charges placed inside or on a circle. This problem has a well defined continuum limit, namely the distribution of charge over a metallic disk. The solution, given in Jeans' textbook², has a finite charge density all over the disk rising to infinity at the edge. Consistency with this limit demands that for increasing N , charges (in the minimum energy state) leave the edge and occupy the interior. The use of a $1/r$ rather than an $\ln r$ potential implies that the circle is really a disk embedded in three-dimensional space and there is thus no contribution with the usual notion that charges reside on the surface of a conductor. These arguments imply that in the case of a sphere, there will be no minimum energy solution with charges in the interior, as is already clear from Earnshaw's theorem³.

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. . . by conducting ellipsoid

SIR—A very old problem in electrostatics is to find the distribution of charge on a conducting ellipsoid which will be taken to be an ellipsoid of revolution. The charge density is of course confined to the surface and is everywhere finite. If we let the length of the axis of rotation go to zero we get a highly idealized two-dimensional object, a two-dimensional (infinitely thin) conducting disk. If the radius of this disk is R , the charge density is well known to be proportional to $(R^2 - r^2)^{-1/2}$. This density

must be a fairly good approximation to the distribution of a number of identical charges (say electrons) placed on the disk. It is not confined to the circumference and it is *not* zero at the centre. Hence there is really nothing unexpected in the results given by Berezin¹ who shows that 12 identical charges distributed evenly on a circle have a greater potential energy than 11 charges on the circle and one at the centre. Perhaps the fact that 12 is the precise number at which one charge is "expelled to the centre" is mildly surprising.

Berezin goes on to conjecture that a similar "spontaneous ejection" of charge to the centre will occur in three dimensions and that this "may lead to some modification of usual theorems of electrostatic stability which claim that at the state of equilibrium all charges . . . are always located at the surface." I think that this conjecture is wrong, and is a nice example of incorrect generalization from two to three dimensions. For example, arguing from two² to three dimensions about the propagation of an impulsive wave emitted from the origin would lead one to the false conclusion that there is a residual disturbance left at the centre in the three-dimensional case.

However, Berezin's letter raises a question to which I have been seeking an answer for some time. It seems clear that it is possible to place an arbitrary number, N , of electrons on a conducting sphere. The question is how do they arrange themselves?

There is little doubt in my mind that they will arrange themselves on the surface, and intuitively the answer would seem to be given by simple symmetry arguments when $N=1, 2, 3, 5$ and $4, 6, 8, 10, 20$, where the second set of numbers are the numbers of vertices of the five regular polyhedra in three dimensions. However, as Berezin has pointed out (in personal communication), such arguments are false in the case where $N=8$. Having the electrons at the vertices of a regular cube has a *higher* energy than when one face is rotated relative to the opposite face by 45° , the distance between the faces being held constant. A quantum treatment of this problem would also be interesting and could also yield mean values of the multipole moments of the system.

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. . . with charged polygons

SIR—The classical electrostatic energy of N point charges, q , symmetrically disposed on a circular circumference with radius R has been recently discussed here (A. A. Berezin, *Nature* **315**, 104; 1985). A surprising feature of these regular poly-

gonal systems was reported for $12 \leq N \leq 400$. Such arrangements are energetically unstable with respect to displacement of one charge to the centre and regular circumferential redistribution of the remaining $(N-1)$ charges. On this basis Berezin made two conjectures: (1) that such a rearrangement is energetically favourable for any large N and (2) that his large- N result indicates a violation of the familiar electrostatic theorem. For a conducting body at electrostatic equilibrium, any net charge must be located at the surface (Faraday shielding).

It should be pointed out that conjecture (2) cannot follow from the large- N polygon results.

If these systems are to model a classical conducting system, their electrostatic potential energy should vary as: $W = \frac{1}{2} cq^2 N(N-1)$ with c a geometrically determined constant (capacitance coefficient). This in turn requires that the electrostatic potential at any vertex location due to the other $(N-1)$ charges should be $U = cq(N-1)$. Evaluation of exact electrostatic potentials for polygons with $N=10^3, 10^4, 10^5$ yields respectively $U=2,238.7970; 29,717.3261$ and $370,466.8178$ (units of q/R).

Clearly the required linear N dependence does not occur. This failure relates to the usual logarithmic distance dependence of electrostatic potentials near linear charge distributions. Thus the polygonal charge arrangements cannot model a conducting system.

For the problem at hand, the potential U may be approximated by exactly summing the contributions of the $2j$ charges closest to a vertex location and treating the remaining charges continuously. For example, choosing $j=18$ and N large:

$$U \approx \frac{Nq}{R} \left[1.112527455 + \frac{1}{\pi} \ln \cot \frac{37\pi}{4N} \right]$$

This expression reproduces the potential energy, U , for polygons with $N \geq 10^3$ with fractional accuracy better than 2.3×10^{-5} ; this accuracy improves with increasing N . The non-linear N dependence is manifest. Using such an algebraic approximation Berezin's first conjecture, the instability of any large- N polygon system, is readily confirmed.

For N surface charges regularly distributed on the surface of a sphere with radius R , the limiting form of the electrostatic energy is:

$$W = \frac{1}{2} \frac{q^2}{R} (N) (N-1)$$

Accordingly for the spherical surface, expulsion of one charge to the centre is energetically possible only in the infinite N limit.

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. . . but for 12 equal point charges?

SIR—Berezin has recently shown that the minimum energy configuration for N equal point charges placed in a circle is different depending on whether N is less than, greater than or equal to 12 (*Nature* **315**, 104; 1985). In the former case ($N < 12$) the configuration of minimum energy is with the N charges at the vertices of a regular polygon inscribed in the circle (configuration A); in the latter case ($N \geq 12$) a lower energy configuration is obtained with one charge expelled to the centre of the circle and $(N-1)$ charges at the vertices of an $(N-1)$ sided inscribed polygon (configuration B). Berezin verified this result up to $N=400$ and claimed it was "very likely" true for all $N \geq 12$. It is easy to show the result does indeed hold for all $N \geq 12$ as follows.

The energy of N charges arranged in configuration A is

$$W_A(N) = \frac{N}{2} \left\{ \sum_{i=1}^{(N-1)/2} \frac{1}{\sin \pi i/N} + 0.5 (N \text{ even}) \right\} \quad (1)$$

and arranged in configuration B is

$$W_B(N) = \frac{(N-1)}{2} \left\{ \sum_{i=1}^{(N-2)/2} \frac{1}{\sin \pi i/(N-1)} + 2 + 0.5 (N \text{ odd}) \right\} \quad (2)$$

It follows simply from equations (1) and (2) that

$$W_B(N) = W_A(N-1) + (N-1) \quad (3)$$

Hence the condition that configuration B gives higher energy than configuration A ($W_B(N) > W_A(N)$) is true for those N where

$$W_A(N) - W_A(N-1) < (N-1) \quad (4)$$

By inspecting the numerical data for $W_A(N)$ only, looking say at the range $2 < N < 40$, it is possible to see that the inequality (4) is satisfied for $N < 12$ as shown by Berezin. It is also easy to see that the left hand side of inequality (4) is increasing faster than $(N-1)$ as N runs beyond 12. Hence the result found by Berezin is true for all $N \geq 12$.

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The rise of 'Mobilism'

Roy Porter

The Dark Side of the Earth. The Battle for the Earth Sciences: 1800-1980.

By Robert Muir Wood

George Allen & Unwin: 1985. Pp.246. £11.95, \$19.95.

"GEOLOGY is dead; long live the Earth sciences". This in a nutshell is the message of Robert Muir Wood's analysis of continental drift from Wegener's chequered career up to the sudden and stunning success of "mobilism" in the plate tectonics revolution of the 1960s.

At one level, this bold survey can be read with equal pleasure and profit as one of the best interpretations of that astonishing scientific upheaval. Unlike Anthony Hallam's *A Revolution in the Earth Sciences* (Oxford University Press, 1973) it does not primarily aim to evaluate the inner logic of successive geological theories; nor does it rival the dramatic blow-by-blow commentary on tectonic breakthroughs offered by William Glen's *The Road to Jaramillo* (Stamford University Press, 1982). But Dr Wood's reading has special virtues of its own. He offers the best account yet of the nineteenth century heritage of speculations about oceans and continents which formed the matrix of drift theory. And he is adroit in bringing his protagonists to life and illuminating their particular parts in the story. Harry Hess is identified as the senior figure who consistently championed younger researchers such as Fred Vine in their heterodox investigations; John Tuzo Wilson is shown to have been the right man in the right place at the right time for propounding the wider "mobilist" synthesis. And due weight is given to the crucial role played by Teddy Bullard as a catalyst, above all in making Anglo-American co-operation in this enterprise so fruitful. Not least, Dr Wood commands a good turn of phrase, and patiently weaves together with enviable skill all the strands of a highly complex narrative.

So here is a sure-footed and wide-ranging account of the triumph of continental drift, with much to offer to specialists and non-specialists alike. But Dr Wood has bigger fish to fry. His case is that the plate tectonics revolution was not just a great transformation in geology — indeed, it is wrongly interpreted if we see it as a revolution in geology. For it was rather a revolution *against* geology.

From its early nineteenth century giants up towards the present, geology's great triumph lay in the stratigraphical and palaeontological vision, in generating the science of the rocks, indeed, a love of the rocks. Geology studied the land masses, especially their mountains. As synthesized in classics such as Eduard Suess's *Das Antlitz der Erde* (1885-1909), it theorized the continents in the context of a

stable, if shrinking, Earth, and then minutely studied their deformations through tireless fieldwork and mapping.

The plate tectonics revolution has left this either wrong or stranded high and dry as an irrelevance. The "mobilist" vision swung attention away from the establishment's preoccupation with hammering out ever more data about the strata. Instead "drifters" turned the whole Earth into their parish and their problem. Unlike conventional geologists, they followed Lyell's advice (think like intelligent amphibians) and gave the ocean beds at least as much attention as the land masses. They insisted on tackling questions, such as the global distribution of the continents, from which orthodox geology shied away; they deployed specialist skills in fields such as seismology and geomagnetics not routinely possessed by pukka geologists; and they ended up proposing a theory which could not be judged — for or against — at the bar of conventional geology.

In other words, the revolution in plate tectonics was the overtaking of geology by an alliance of disciplines which we may call geophysics. Or, put another way, enter the Earth sciences, marching under the banner of a global theory, which rendered old geology obsolescent in much the same way as Darwin's evolutionism left behind classificatory natural history as a key to the economy of life.

This was a revolution made by aliens. Some were geographical outsiders, such as the South African, Alexander Du Toit, or Warren Carey from Australia. Others were disciplinary marginal men, classically of course Wegener himself (first and foremost a meteorologist, as Dr Wood rightly insists, while perhaps unduly playing down his geological credentials). Practically all the pioneers of "mobilism" had training or research experience in some branch of geophysics — often in seismology or geomagnetism — which set them apart from true-blue geologists. Not least, "mobilism" was typically the brainchild of the "mobile", the young and ambitious, and was perceived by the old guard to be unsettling (in America it was dubbed "leftist", in Russia, "bourgeois").

Thus the plate tectonics revolution did not emerge out of the geological main stream. Above all, its inspiration came from that late arrival, deep-sea geology. And this in itself would have been unthinkable without the gigantic sea-bed surveying operations funded at huge expense by allied governments during and

after the Second World War (projects in which Hess, Ewing and Bullard were all prominent); and then, at a later stage, without the United States pouring money into seismology to monitor nuclear tests. Thus strategic needs produced new fields of expertise; these led to the discovery of the magnetic field anomalies of seafloor rocks, which in turn proved the surprising youth of the ocean floor, and gave rise to the key idea of seafloor spreading, that *since qua non* of the vision of continents borne on mobile plates.

Of course, Dr Wood is not arguing that conventional geology contributed nothing at all to this. But the fact that even so catholic and prescient a geologist as Arthur Holmes got so far yet no further in convincing himself and others of drift, perfectly shows its limitations even at its best. And, at its worst, orthodox geology proved to be an ostrich.

So plate tectonics should not be seen as a revolution in geology, but as marking geology's eclipse, a refocusing of scientific attention away from the rocks towards the whole Earth, a re-think of planet Earth for the space age. Science thus advances, Dr Wood argues, not by accumulating data, nor even by internal theory switches, but by massive re-groupings of disciplines. The old gods are devoured not by their own children but by their neighbours' (except perhaps in the Soviet Union where geological gerontocrats have had more success in keeping the new "drifters" at bay). Late Victorian geology had resented being put in its place by the physico-mathematical imperialism of Lord Kelvin, and had unilaterally declared its independence of physics. Now, a century later, geophysics has had its revenge.

Occasionally, the contrast Dr Wood draws between senile geology and virile Earth science is overstated for polemical effect (his own evidence sometimes gives it the lie); and it is a pity that, drawing too freely upon hindsight, he oversimplifies certain complex figures, such as T.C. Chamberlin and Maurice Ewing, and turns them into whipping boys. Although providing attractive line drawings of the main protagonists, the book would have benefitted, I think, from some explanatory diagrams. Yet all praise to him for recognizing that in understanding the history of science no less than in understanding the history of the Earth, "fixism" will not do. No more than the continents, are the sciences themselves fixtures; they too are subject to drift, expansion and subduction. This is an important re-interpretation, whose "mobilist" vision should send some shock waves through the geological establishment. □

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Getting down to the bare bones

Richard Potts

The Analysis of Animal Bones from Archeological Sites. By Richard G. Klein and Kathryn Cruz-Uribe. University of Chicago Press: 1984. Pp.266. Hbk \$19.95, £18.95; pbk \$8.95, £8.50.

INVESTIGATION of past human activities and ecological settings requires many analytical steps and cautious weighing of the processes that transform living phenomena into buried, fossilized traces. Archaeological faunal remains—one such “end product” of this transformation—have provided extremely important, albeit controversial, evidence about early hominid behaviour and environments. After determining the species, body part, sex and age of death of the animals represented by bone specimens, zooarchaeologists often attempt to estimate the relative number of individuals or bone units of each species found in an accumulation of bones; further, they may construct mortality/age distributions for well-represented species. These and other quantitative data help to test ideas about environmental change; catastrophic versus attritional death of animals; whether humans, carnivores or geological agents were responsible for bone aggregates; and whether humans hunted or scavenged, and how they selected certain species and body parts.

The theme of this volume is the entire process by which zooarchaeologists interpret the past from the assemblages of vertebrate bones often found in

archaeological excavations. The first of two sections introduces lucidly the fundamentals of animal bone identification, faunal data collection and analysis. The second provides computer programs in BASIC that generate data used widely in faunal analysis (minimum number of individuals, age distributions and so on). These programs are explained clearly and the procedures used to quantify bone assemblage characteristics are discussed thoroughly. Inferences about ancient people, their sites and environments are illustrated largely from Klein's own research on prehistoric faunas of southern Africa and Europe. Thus, the book provides an excellent summary of the approaches Klein has developed.

In illustrating certain aspects of faunal analysis, the authors offer interpretations that appear *post hoc*, without due consideration of alternatives; this will disquiet some researchers. Others may feel that the authors' distinction between the comparative and taphonomic approaches to faunal studies is unwarranted, especially since the latter approach (which examines how modern processes create faunal patterns) is played down.

These potential points of dispute do not overshadow the book's value, however. It will be widely used as a text on fundamental aspects of faunal analysis and as a source of ideas and useful programs for generating data that help to explore the subtle, bony traces of ancient human activities and environments preserved in the geological record. □

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The wonderful sky at night

Martin Harwit

The Cambridge Atlas of Astronomy. Edited by Jean Audouze and Guy Israël. Cambridge University Press: 1985. Pp.432. £29.95, \$75.

How wonderful to open this volume to find an absolutely lavish collection of new drawings, charts, explanatory diagrams, illustrated tables and photographs—not just of astronomical objects, but also of terrestrial fossils and atmospheric and volcanic structures.

There are charts and maps of all kinds, and pictures of the lunar surface and of all the planets and satellites visited by spacecraft. Some of these we have seen before, but most of them are selected to be instructive: tracks left on the Moon's surface by the Apollo lunar vehicle, along-

side lunar dust, highly magnified, showing an occasional spherical droplet, and next to that an electron micrograph of a single grain showing tracks of solar cosmic rays that have bombarded the lunar regolith leaving a record of solar activity over past aeons. Over the page we find a picture of a micrometeorite, followed by views of slices of larger meteorites and embedded ion tracks. Just by looking at such sequences readers will gain insight into the nature of lunar surface material, meteoritic matter and some of the processes to which both have been subjected. In turn, they will better appreciate the way a knowledge of these processes helps us to understand the past histories of the materials which make up the Solar System.

This, then, is a beautifully produced book of great pedagogic value. Amateur astronomers, high school students and professionals will find here a visual display which is both arresting and informative. What I am about to say should, therefore, not discourage one from investing in this volume.

My particular disappointment started when I stopped looking at the illustrations and began reading the text. In just the first hour of browsing I noted a dozen flaws of all kinds. There are numerous typographical errors, and the index appears to have been compiled with insufficient care over the cross-referencing, which is so important in a volume such as this. More specifically, a figure caption (p.11) describes the telescopes used by Galileo, and adds, “The principles of these telescopes were conceived in Venice by an unknown genius”. Actually, these instruments first surfaced in the Netherlands, late in 1608, roughly a year before Galileo began his observations. And on p.387 we find the statement: “The existence of neutrinos was proposed for the first time by Paul Dirac on a theoretical basis to explain the conservation of the angular momentum, or spin of the ensemble of particles produced by the beta disintegration of the neutron”. Actually it was Wolfgang Pauli, with some help from Enrico Fermi in naming the particle, who conceived of the neutrino for conserving energy in beta decay.

More serious, however, is the question of purpose. How much should the text explain—couldn't the occasional bit of calculus have been avoided through use of a simple diagram? One has the impression that the editors wanted to pack every possible concept into this single volume—massive neutrinos, the number of neutrino generations, proton decay and other current exotica. This has taken its toll on clarity. For example the section on cosmology begins by listing five principles alleged to guide the construction of cosmological models: “The principle of uniformity, the cosmological principle, the anthropic principle, the principle of equivalence and Mach's principle” (p.381). But Mach's principle still does not fit into any of our current theoretical models; and the anthropic principle merely tells us to disregard theoretical models which don't ultimately produce cosmologists. Everything seems to have been crammed in with little regard for separating the important from the frivolous.

Still, the editors and publisher have done remarkably well with the illustrations and, on balance, I recommend the *Atlas* highly. I just hope that the text can be thought through once more, in time for a well-deserved second edition. □

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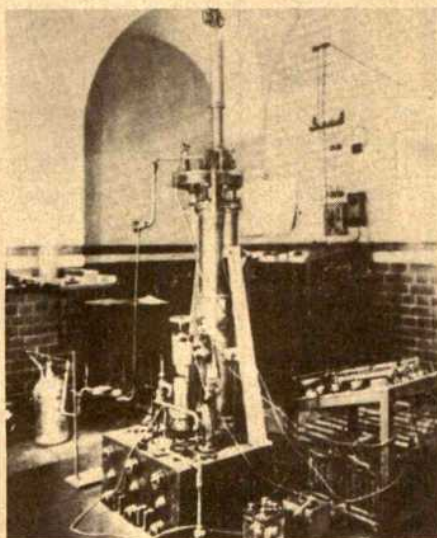
- *The Dawn of Animal Life: A Biohistorical Study* by Martin F. Glaessner. Cambridge University Press. £9.95, \$19.95.
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The unseen revealed

A. Howie

The Beginnings of Electron Microscopy. Edited by Peter W. Hawkes. Academic: 1985. Pp.633. \$88, £67.

DURING the 1920s, the work of Busch and Gabor on the focusing action of short magnetic solenoids in the high speed cathode ray oscillograph, and the separate, celebrated discoveries in electron diffraction, sparked off an idea which some minds — with a faith reminiscent of a mediaeval cathedral-builder — took seriously. That idea was the construction



The Met-Vick EMI, the first commercially produced electron microscope. Installed at Imperial College, London in 1936.

of microscopes to surpass the resolution of optical instruments by as much as five orders of magnitude, a limit deduced purely from the electron wavelength.

Here, in partially coherent illumination from a variety of authoritative sources (delightfully illuminated themselves by Cosslett), one can learn how the idea survived the technical obstacles of power supply instability and poor vacua, as well as the deeper problem of lens aberrations. It seems that other difficulties, such as the slump of the early 1930s and the Second World War (with enemy occupation of Holland and France to be borne by Le Poole, Grivet and Dupouy), were only marginally more serious than the general indifference of optical microscopists. Did they simply exaggerate the beam damage problem? Or were they more realistic about the looming difficulties of specimen preparation and image interpretation described here by Drummond, Hall and Fernandez-Moran?

In this enjoyable collection of reminiscences, Dr Hawkes has unfortunately missed some potentially important contributions because of illness, death or prior publication elsewhere. The brilliant construction by Prebus and Hillier, two

graduate students at Toronto, during four months in 1938, of an instrument eventually achieving 100Å resolution is only briefly mentioned. Less serious is the absence of a major contribution from Ernst Ruska (whose own book, *The Early Development of Electron Lenses and Electron Microscopy*, appeared in English translation five years ago). His towering achievement is manifest here from different viewpoints. As noted by Mulvey, the transmission microscope producing today's images at atomic resolution follows in all crucial respects the original Ruska design. Restricted, however, by lens aberrations to the small-angle range where coherent scattering effects dominate, the instrument depends on a marriage of electron optics and electron diffraction far closer than anyone then envisaged. Although the reader may trace in Le Poole's account the instrumental foundations of that partnership (also laid by Boersch), sample its fruits in Dupouy's magnificent high-voltage images or sense with Hibi the holographic future, he must turn elsewhere (for example to *Fifty Years of Electron Diffraction*, published by Reidel in 1981) to appreciate the enormous benefits which the union brought to both disciplines.

As its originator von Ardenne describes, scanning electron microscopy provides a contrapuntal theme even though it shares the same origins with the more conventional transmission method. A much better understanding of electron beam scattering processes is required, however, to exploit fully the rich range of imaging modes available in scanning. Its wide application depends more on ease of specimen preparation and depth of field than simply on resolution. The post-War exploration of these questions and the development of instruments for manufacture are recounted by Oatley and his colleagues. This contribution, as well as Mulvey's, covers two of the three principal manufacturing initiatives in electron optics where, contrary to received doctrine, British industry exploited an idea originating elsewhere. Le Poole and Kanaya treat the early stages of the important Dutch and Japanese electron optical industries.

A third theme, dating from pre-War Germany but little mentioned here, is the field emission microscope. Closer to the simplicity of the Leeuwenhoek microscopes than anything else in electron optics, the field emission tip has fathered the recent spectacular advances in scanning transmission and in scanning tunnelling microscopy. Before long, perhaps, Dr Hawkes will edit a second volume rediscovering in this more recent progress our great debt to the persistent vision of the early pioneers. □

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Plumbing the depths

Stephen Moorbath

Archaeo Geochemistry: The Origin and Evolution of the Archaean Continental Crust. Edited by A. Kröner, G. N. Hanson and A. M. Goodwin. Springer-Verlag: 1984. Pp.286. DM87.

UNTIL recently, the Archaean (c. 3,800–2,500 Myr ago) was still a great unknown in the earth sciences. This situation has changed dramatically since the 1960s as a result of two main factors: (i) advances in isotope dating methods and their application to Precambrian rocks, and (ii) the recognition that the oldest exposed gneiss and greenstone terrains retain a wealth of primary igneous, sedimentary and geochemical features.

It was clearly difficult to find an accurate title for a book which contains 13 miscellaneous contributions to a wide field, ranging in style from reviews to research papers and arguably more suited for publication in journals. Thus the principles of geochemistry were no different during the Archaean than they are today. Some workers (including myself) even consider that Archaean continental crust originated and evolved by processes closely similar to those of geologically recent times, albeit much more rapidly because of greater terrestrial radiogenic heat

The Ecology of Rocky Coasts

Edited by Dr P G Moore and Dr R Seed

This is probably the most comprehensive volume on the subject of the ecology of rocky coasts ever published. The book is the work of 28 internationally outstanding experts in the field and includes the most recent research and evaluations in this increasingly important area. It provides a complete overview of littoral and sub-littoral communities which will be of immense value to ecologists and marine biologists.

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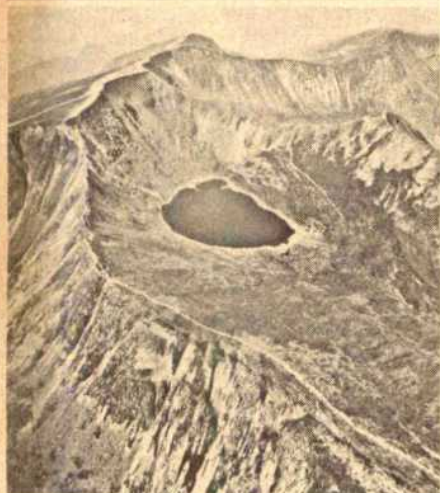
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production. The deep crustal level represented by many Archaean rocks now exposed at the surface conveniently gives us insight into the chemical and petrogenetic processes occurring in the deeper reaches of the continental crust, not normally observable at those accreting or colliding plate margins where young continental crust is nowadays being created or reworked.

Certain aspects of the subject are efficiently dealt with in this useful volume, whose stated aim is to summarize some of the main issues in the broad geochemistry of Archaean rocks which were addressed during a nine-year stint of one of the projects ("Archaean Geochemistry") of the International Geological Correlation Programme. Topics range from the accretion history of the Earth and composition of the primordial mantle to greenstone belt evolution, petrogenesis of komatiites, the nature of high-grade gneiss terrains and the role of fluids in their development and geochronology, and the relation of Archaean sediments to the overall composition of the Archaean continental crust. Also included are geochemical and geochronological data on regions in the Soviet Union, China and India which were hitherto little known in the Western literature. It is refreshing, for once, to see some reliably documented new isotopic age data from the oldest shield areas of the Soviet Union to which up-to-date analytical and interpretative techniques have been applied.

I regret the absence of a contribution on the rapidly developing field of biogeochemistry and related topics, since the Archaean saw the transformation of "primordial soup" into the highly organized, far-reaching second course. Some discussion of Archaean metallogenesis would also have interested geochemists. □

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Red Tam nestling in the Helvellyn corrie. From *Discovering Landscapes in England and Wales* by Andrew Goudie and Rita Gardner. Published by George Allen & Unwin at £12.95.

Animal cognition

Geoffrey Hall

Animal Intelligence. Edited by L. Weiskrantz. Clarendon: 1985. Pp. 223. £32.50.

THE publication of this work (the proceedings of a Royal Society discussion meeting) just over a century after the appearance of *Animal Intelligence* by G. J. Romanes tempts one to make comparisons. Romanes was interested in animal intelligence because he felt the findings of comparative psychology had as much relevance to Darwinian theory as did those of comparative anatomy.

Romanes would be at home with Jerison's contribution to the present volume, a chapter which charts the evolution of encephalization and the implications of brain size for mental activity. But, as Jerison points out, little use is made elsewhere of evolutionary theory in organizing the wide range of facts about animal behaviour presented by the various contributors. One of the reasons for this neglect may be discerned in the chapters by Macphail and by Mackintosh and his collaborators. Although Romanes felt able to rank the various animal groups according to their level of intelligence (his scheme is reproduced in Weiskrantz's introductory chapter), modern experimental studies of animal learning (such as are reported by Mackintosh) have found precious few differences between species that can be unambiguously interpreted as being the result of differences in intelligence. Macphail concludes that we might as well accept what he calls the "null hypothesis" — that extant non-human vertebrates do not in fact differ in intelligence.

Romanes has been castigated by subsequent comparative psychologists for basing his conclusions on mere anecdote rather than on careful observation and experiment. To some, Macphail's conclusion may seem not so much an accurate judgement of the intelligence of animals as a condemnation of the skills of modern experimenters. And certainly, some of the most striking examples of intellectual achievement among animals reported in this book come not from laboratory studies but from observations made in natural or semi-natural conditions. Goodall concludes her intriguing survey of the innovative behaviour displayed by the chimpanzees of Gombe with an assertion of the value of (carefully documented) "anecdotal" reports of natural behaviour.

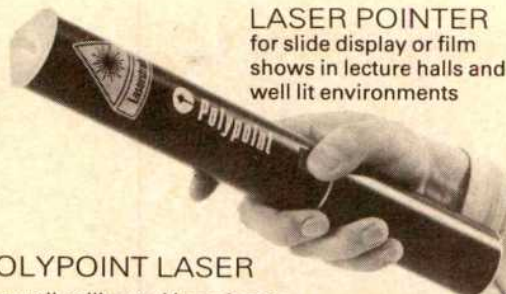
However this may be, none would deny the need for experimental analysis in any attempt to determine the mechanisms responsible for seemingly intelligent behaviour and much of this book is devoted to reports of such work. Several chapters present accounts of the behaviour shown by primates when solving the problems set for them by experimental psychologists. It

is made clear that their behaviour cannot be interpreted simply as the acquisition of new habits and must require intelligence of a higher order. But perhaps this has never really been in doubt. More significant, therefore, is the elegant analysis presented by Dickinson of the mechanisms by which that least esteemed of animals, the laboratory rat, learns the simplest of tasks, that of pressing a lever to obtain food. What emerges is a need to allow the rat knowledge about the relationship between its actions and its desired goals, and credit it with the ability to carry out inferences on the basis of that knowledge. No more than this is claimed for the primates, a fact that not only speaks in favour of Macphail's "null hypothesis" but also helps to justify the belief that has sustained so many studies of animal learning — that the study of cognitive processes in one vertebrate species will reveal principles of intellectual functioning that have general applicability.

It would be a disappointment if such general principles as emerge were to be found not to apply to our own species. But the possibility remains that human intelligence is different in kind from that shown by non-human animals, being based on our possession of a form of language that is beyond the capacity of any beast. Empirical investigation of this hoary notion long seemed impossible. But perhaps the most interesting implication of the recent attempts by several experimenters (Terrace, Gardner and Gardner, Savage-Rumbaugh and others are represented in this book) to teach a form of human language to an ape is that their work appeared to allow just such a possibility. Unfortunately, the outcome has been inconclusive. There is no real consensus as to what the apes have actually achieved or, more worrying, as to what it is that they would have to do in order for us to be sure that they had learned a language. An empirical solution seems as far away as ever.

More generally, this book leaves one with the impression of a field of study that is productively active, has achieved much in the hundred years since Romanes and is likely to achieve more in the near future. And this is only half the story. Romanes devoted roughly half of his book to the intellectual achievements of invertebrates. That these animals receive no mention in the present volume is its only serious failing. It is now becoming clear that the application of methods of study and analysis previously applied only to vertebrates can reveal evidence of equivalent intellectual sophistication in invertebrates. Our rapidly advancing knowledge of invertebrate neurophysiology fosters the hope that a comprehensive account of the biology of intelligence is almost within reach. □

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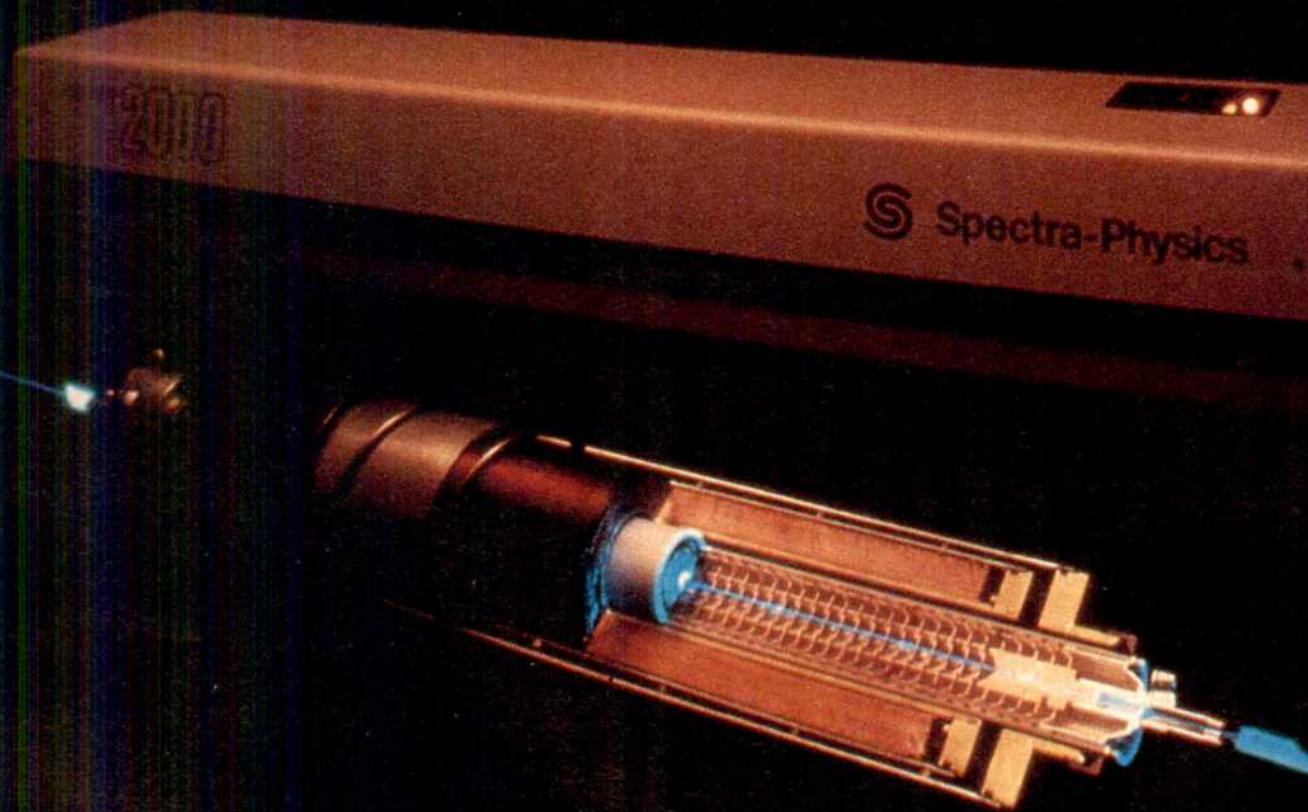
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Birth of the maser and laser

Shortly before his death in January 1984, Professor Alfred Kastler, winner of the Nobel Prize for physics in 1966 for his work on optical pumping, submitted to Nature a book review of Masers and Lasers by Mario Bertolotti¹. Published here is an edited and abridged version of the review.

THE word 'maser' was coined by Townes in 1951, as the acronym of 'microwave amplification by stimulated emission of radiation', and 'laser' was formed in the same way, 'light' replacing 'microwave'. In their famous *Physical Review* paper of 1958², Schawlow and Townes had used the term 'optical maser', a linguistic nonsense since it would literally mean 'optical microwave amplification...'. Incidentally, the most valuable property of these devices is not amplification, though they are widely used for this purpose in the microwave and optical regions, but self-sustained oscillation, so we should arguably call them 'mosers' and 'losers'!

The subject is, however, much older than the maser and the laser. Its prehistory began in 1916-17 with two papers by Einstein^{3,4} in which the distinction between spontaneous and induced emission of radiation was made. Both processes were shown to occur not as spherical waves, familiar from Maxwell's theory, but as a highly directional form of radiation (*Nadelstrahlung* rather than *Kugelstrahlung*, as Sommerfeld put it in 1922), the spontaneous emission having the same direction as the stimulating light and accentuating its intensity (negative absorption). The emission cannot, however, be in phase with the stimulus, for this does not permit conservation of energy; instead, the atomic emitters must be a quarter-period ahead of the driving field, as experimentally verified by Meslin. Einstein made no mention of the coherence of the stimulated emission, and it is tempting to speculate that this delayed the invention of the laser.

The exchange of momentum between atoms and light is clearly crucial and Einstein had examined this in great detail as early as 1909. As a working hypothesis, he suggested that light may be regarded as particles (our photons), which are to be thought of as local singularities in which the electromagnetic field energy is concentrated. These 'particles' are guided by the electromagnetic wave, interacting as they proceed. In Einstein's mind, therefore, wave properties such as interference and diffraction are a collective property of these particles. The richness of this idea in studies of both light and charged particles cannot be overstressed.

The important connection between the absorption and emission of light and the phenomenon of anomalous dispersion is perhaps not quite so obvious. The dispersion formula of Kramers and Heisenberg (1925)⁵ in fact contains 'negative dispersion' terms, which are connected with 'negative absorption' or, in other words, stimulated emission. The existence of these terms was demonstrated experimentally by Ladenburg and co-workers; in particular, Ladenburg and Kopfermann^{6,7} found that on raising the current density in a neon discharge, the negative dispersion terms grew more important, the temperature corresponding to the population ratio of the energy levels involved reaching values far higher than the kinetic temperature of the atoms and electrons. Had these authors continued their investigations and extended them to argon, they would probably have attained population inversion (a prerequisite for laser and maser action, to which I will return later). As Bertolotti mentions, Schawlow later thought it likely that Ladenburg and Kopfermann "did not continue their studies on anomalous dispersion because they believed so firmly in equilibrium that they thought it was impossible to go so far away from it as to have negative absorption"⁸.

In the 20 years that separated the 1930s—by which time the concepts of stimulated emission and negative absorption had become well established—and the invention of the maser and laser, the problem of magnetic resonance was solved and the process of optical pumping introduced. Although not directly relevant, the knowledge acquired was later useful in the development of lasers. Incidentally, Bertolotti's short note on the history of the classical theory of magnetism fails to mention Pierre Curie's thesis on the magnetic properties of matter, completed in 1895; he also wrongly credits Pauli with developing the concept of 'spin' in 1920 (see box), but the complex history of the different types of magnetism demands a fuller account than is given in the book.

In the early 1950s, prehistory ended and history began. The maser principle was developed independently at Columbia University (New York) by Townes, at the Lebedev Institute (Moscow) by Basov and Prokhorov and in the University of Maryland by Joseph Weber. It was at a conference in Ottawa in 1952 that Weber first mentioned publicly the principle of the maser, the text of his lecture appearing in print a year later⁹. The work of Townes and colleagues on the ammonia maser appeared in 1955¹⁰, a year after the first publication on the subject by Basov and Prokhorov, who, as Bertolotti notes, "pointed out the theoretical possibility of a device producing microwaves by using stimulated emission at an All-Union conference on radiospectroscopy in May 1952, but ... their first written paper was not published until October 1954"¹¹.

To generate coherent electromagnetic radiation, three conditions must be satisfied: stimulated emission, strong enough to surpass the absorption of the medium, must be obtained; a suitably inverted population must be created; and a convenient cavity must be used. In fact, the first two conditions are identical. As far as the last one is concerned, the cavity in the microwave region is just a suitably shaped metal box. The latter usually has the form of a cylinder closed at one end by a piston so that the proper mode of oscillation can be matched to the frequency of the atomic or molecular transition. Single-mode operation is easily achieved.

In the case of lasers, the appropriate cavity is a Fabry-Perot interferometer, as Schawlow and Townes pointed out in their fundamental paper of 1958², and if any French contribution to the laser deserves mention this is it. Fabry and Perot developed their instrument at the University of Marseilles in the early years of the century¹². The cavity of this device is cylindrical and the diameter of the two parallel circular faces is conventionally greater than their separation. Here I should digress briefly to say a word about R. Boulouch, who was a precursor of Fabry and Perot. In 1893 he published a description in the *Journal de Physique*¹³ of the very fine fringes he had obtained with thin silver layers, and mentioned that, for a suitable value of the distance between the reflecting surfaces, he had been able to separate the components of yellow sodium light: "Les anneaux dédoublés apparaissent, pour une épaisseur convenable de la lame, singulièrement nets" (for a suitable choice of the thickness of the plate, the two ring systems are remarkably distinct); he also noted that the line-shape could be explained in terms of the Airy function. Boulouch was at that time a grammar-school teacher at the Lycée of Bordeaux, where I myself taught in the years 1929-31, and where I met the elderly Boulouch, by then

Kastler's views on Pauli's contribution

BERTOLLOTTI is mistaken when he attributes the development of the concept of 'spin' to Pauli in his 1920 paper. This paper deals with the quite different problem of paramagnetism. 'Electron spin' was in fact discovered in 1925 by Uhlenbeck and Goudsmit. Why did this concept become necessary to atomic physicists? The extensive study of fine structure of spectral lines and the related anomalous Zeeman effect obliged physicists to introduce a new quantum number to stand alongside the principal quantum number n , which determines the energy of an atomic state, and the azimuthal quantum number k , which gives the angular momentum of a state, both in units of Planck's constant h and both being integers. (Today we use l where $l=k-1$.) The third quantum number, which we represent by the letter j , sometimes takes a half integer value—this came as a surprise at the time—the vector representing the total angular momentum of the atom.

In a lecture given in Germany in 1965, when he was awarded the Planck medal, Samuel Goudsmit gave his personal version of the discovery of 'electron spin'. He cited a passage from a letter sent to him in March 1926 from Copenhagen by his friend and colleague L. H. Thomas. In this letter Thomas writes: "I believe that you and Uhlenbeck had the good luck to see your spinning electron published and discussed before Pauli knew of it. More than a year ago Kronig had conceived the rotating electron and had developed his idea; Pauli was the first person to whom he showed this paper. Pauli told him that it was a ridiculous assumption, and so it happened that the first person to see Kronig's paper was also the last one." And Thomas concludes the story by stating: "All this shows that God's infallibility is not extended to whom had nominated himself to be his vicar on earth." German

physicists have since composed the rhyme: "Der Kronig hatt 'den Spin entdeckt, Hatt' Pauli ihn nicht abgeschreckt." (Kronig would have discovered spin if Pauli had not discouraged him.)

There is another reason why Goudsmit adopted a very critical attitude towards Pauli. In his Nobel lecture of 1945 Pauli gave an explanation of the hyperfine structure of spectral lines by postulating the existence of a nuclear spin whose magnetic moment interacts with the moment of the electron cloud, an idea which he had published in 1924. He claimed that this publication had influenced Goudsmit and Uhlenbeck in their claim of an electron spin. Goudsmit maintained that Pauli had never considered an internal moment of the nucleus. He had especially not foreseen it for the proton. For him the nuclear angular momentum, with its small associated magnetic moment, was due uniquely to the orbiting electrically charged particles inside the nucleus, namely the protons and, as was assumed at that time, the electrons. So the discovery of electron spin as an internal momentum owed nothing to Pauli.

Pauli, in his young years, had been a severe judge for the physicists of his generation who feared his sharp criticism, and as we have seen his influence on the development of physics was not always a positive one. I made his personal acquaintance some years before his untimely death, meeting him at the Physics Summer School of Les Houches and in Varenna. In those days he had become a friendly and indulgent person. His exclusion principle, on which are built Fermi-Dirac statistics and the explanation of the shell structure of the electrons in an atom, remains a great achievement in the development of physics. □

retired. During 1889 and 1890, Charles Fabry had also taught at this same lycée, and had been a colleague of Boulouch, but he never mentioned the priority of Boulouch's researches. So far as I am aware, only Tolansky cites Boulouch's work, and he gives the wrong reference (1906 instead of 1893)¹⁴.

Let us now consider how population inversion was obtained. In Townes's first ammonia maser, molecules were excited into a higher energy state by means of a molecular beam, the molecules being separated by an inhomogeneous electrical field. Many celebrated experiments have used this atomic beam technique, but how many people remember that it was invented by Louis Dunoyer in Paris in 1919? Another elegant method of obtaining population inversion in the microwave region was exploited in Bloembergen's three-level maser. Some six different procedures have been used to trigger off laser action; it has often been found surprising that a simple gas discharge can create the necessary non-equilibrium state, as it does in the argon laser. Among the other methods, we recall electron impact, chemical reaction and Javan's proposal of 1959, which led to the first gas laser able to operate continuously, using two gases, and which was subsequently exploited in the high-power CO₂ laser. Bertolotti discusses all of these techniques thoroughly in Chapters 4, 5 and 6 of his book.

Finally, optical pumping can be used to obtain population inversion and it was in this way that Maiman operated the first laser, relying on the chromium ions in a ruby crystal¹⁵. Perhaps I can give my own version of the story. Optical pumping techniques were developed in my Paris research group during the period 1950-70 by a dozen young and enthusiastic research students under the guidance of Jean Brossel. It was this collaborative work that was the origin of my Nobel prize. In the very first paper in the new journal *Applied Optics*¹⁶, Francis Bitter surveyed this development and Jean Brossel has also given us his view of the story¹⁷. Ground-state optical pumping had been preceded by the double-resonance method, in which optical resonance produced by light is combined with magnetic resonance generated by a radiofrequency coil. As Bertolotti mentions, the Italian physicists Fermi and Rasetti had applied an oscillating magnetic field to atoms at optical resonance as early as 1925

and observed an oscillating Hanlé effect (depolarization of the resonant light). In his *Note e Memorie di E. Fermi*¹⁸, quoted by Bertolotti, Rasetti describes how

Fermi pointed out that, since the mercury resonance line showed an anomalous Zeeman effect with a Landé factor of 3/2, the mercury atom should more likely precess with a frequency 3/2 times higher than the Larmor frequency. The choice between the two alternatives might be decided by investigating the behaviour of the polarization under magnetic fields, of the intensity of about one gauss and a frequency of a few megacycles per second in approximate resonance with the precession frequency of the atom.

It thus seems that in 1925 the idea of Brossel's double-resonance experiment had already occurred to Fermi, but the fields applied (1-2 G) were insufficient to produce any appreciable resonance.

Brossel and I arrived at this method independently after an exchange of letters about the failure of attempts to investigate an excited state of mercury by Bitter's method; Brossel was working in Bitter's laboratory at Massachusetts Institute of Technology (MIT) at the time (1949). We published the principle together¹⁹, after which Brossel went on to perform the corresponding experiment and published the result. In my 1950 paper²⁰, I suggested altering the population of the magnetic m -states in the ground-state of the atoms by irradiating them with polarized light; if the polarization was linear, a change would be produced between states of large and small m (alignment), while if the polarization was circular (left- or right-handed), concentration of the atoms in either the negative or positive m -state (polarization magnetization of the ensemble of atoms) would be favoured.

As a young student, I had the opportunity of visiting Germany in 1922, where I bought Sommerfeld's book. I read it eagerly, being especially interested in the section on the "Auswahlprinzip und Polarizationsregel" ("selection principle and polarization rule") in which the thesis of Sommerfeld's Polish research student A. Rubinowicz is summarized. Optical pumping is just an application of this principle and rule.

In 1951, Brossel returned to Paris after six years abroad, three with Tolansky in Manchester and three with Bitter at MIT.

(British and American scientists may find it difficult to imagine the state of scientific isolation in France during the war years; we were cut off from the Allies and had no desire for contact with German scientists.) Brossel had a good idea of the progress of science abroad and knew what equipment we should need to resume our research, essentially oscillators and photomultipliers to develop the double resonance and optical pumping techniques. Our expenses were defrayed by the French Centre National de la Recherche Scientifique.

I am grateful to Bertolotti for recalling that we never intended to work on stimulated emission. Our aim was to change not the energy but the angular momentum of atoms, either in their ground state or in their excited states, thereby detecting magnetic resonance transitions which could be measured with high precision. My Nobel prize was given for the development of the optical methods of radiofrequency resonance (the 1964 Nobel prize in physics having been awarded to Townes, Basov and Prokhorov, the pioneers of the maser and laser). Despite this, my photograph appeared on the cover of a weekly journal with the caption "the father of the laser". I refuted this immediately in an address before the French Academy of Sciences, but there seems no way of suppressing this *canard* which continues to reappear intermittently. I deeply regret that Jean Brossel did not share the Nobel prize with me.

Bertolotti continues with an account of recent developments, the construction of the free-electron laser for example, and in

a concluding chapter examines the statistical properties of light, which are very different for coherent and incoherent radiation; this material is more demanding mathematically than the earlier parts of the book.

It is a pity that there was not room to give a more popular account of the applications of lasers and masers, which have become marvellous tools, in physics of course, but also in metrology, astronomy, medicine and industry, not forgetting the laser light that reads compact disks. The author alludes only briefly to such applications, observing that the field is "still open today"—perhaps the best applications are still to come? The role of the laser in turning Gabor's proposal of holography into a reality is briefly mentioned. Let us hope that the newer high-power lasers will never be used as weapons in outer space. Lastly, a word to the publishers: it would have made things much easier for the reader if the notes and references had been grouped at the end of the book, instead of having to be hunted for at the end of each chapter.

Let me not end on a negative note, however. Professor Bertolotti's book is a fascinating account of the history and prehistory of the maser and the laser. Most readers will enjoy his thumbnail sketches of the scientists involved, for his book tells us not only about physics but also about physicists, a very attractive feature. Although it is not aimed at the non-scientist, scientists from other fields will be able to follow the story since complicated mathematical reasoning is usually avoided.

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High-power solid-state lasers

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Laser power has increased by 11 orders of magnitude since Maiman's first demonstration of 10^3 W in 1960. Present high-power solid-state laser systems have been irradiating targets with 1–10 terawatts of power in 1-ns pulses at wavelengths varying from 1.05 to 0.26 μm . The Nova laser is beginning experiments in the 10 to 100-terawatt range. There are numerous possible means of extending solid-state technology to the 500 to 1,000-terawatt level and to efficient, high-average-power operation.

THE prospect of constructing lasers having peak powers greater than 10 TW excited the interest of experts in thermonuclear reactions in the early 1960s¹. This power, when focused to intensities of $\sim 10^{14} \text{ W cm}^{-2}$ onto small capsules containing deuterium and tritium fusion fuel, was thought to be capable of generating thermonuclear reactions^{2,3}. Other applications considered at the time included the optical pumping of X-ray lasers, and equation-of-state and other atomic-physics measurements at high temperature and density. Efforts to construct lasers powerful enough to demonstrate these concepts have been extremely successful (see Fig. 1): thermonuclear conditions in deuterium-tritium (DT) fuel are being attained⁴, stimulated emission at soft-X-ray wavelengths has been observed^{5,6}, and a variety of other measurements are being accomplished⁷.

Laser power has increased by 11 orders of magnitude, from Maiman's 1960 Q-switched ruby laser⁸, which produced ~ 1 kW, to the Nova laser, which can produce up to 100 TW (Fig. 2). In the 1960s the invention of mode-locking led to the generation

of laser pulses as short as 10^{-11} s, and Nd:glass gain media capable of amplifying these short pulses to moderate energies (~ 100 J) were developed. The appropriate combination of these technologies yielded lasers producing nearly 100 GW. The physics of these systems was incompletely understood, however, and while laser-target experiments were enticing, the results were erratic. By 1971, programmes were conceived to build 10-TW lasers to demonstrate inertial confinement fusion (ICF) concepts at spatial scales large enough to ascertain the potential for generating efficient thermonuclear burn⁹.

Inertial confinement fusion research

In 1971, three laser media—atomic iodine, CO_2 and Nd:glass—were available for scaling to laser sizes capable of producing 10 TW (that is, 10 kJ of energy in pulses shorter than 10^{-9} s). The atomic iodine laser system was not chosen for development in the national laboratory programmes in the United States because the diagnostics were somewhat difficult at the laser

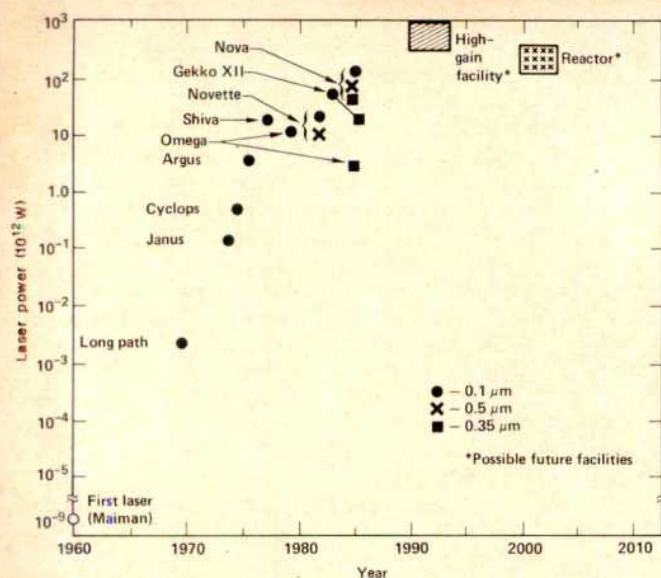


Fig. 1 Laser systems have increased dramatically in peak power since 1960. The laser names before 1980 are Livermore systems although other laboratories contributed significantly to this progress. Lasers in the 10-TW category were Shiva and Novette, the two-beam precursor to the 10-beam Nova system. Gekko XII, a 12-beam laser delivering 50 TW at $1\ \mu\text{m}$ or 20–50 TW at $0.5\ \mu\text{m}$, is beginning experiments at Osaka University. Omega is a 24-beam (12-TW, $1.0\text{-}\mu\text{m}$) laser system at the University of Rochester, recently converted to 4 TW at $0.35\ \mu\text{m}$. The high-gain and reactor facility boxes show the objectives of high-power laser design.

wavelength of $1.3\ \mu\text{m}$ and because there was little experience in generating sub-nanosecond laser pulses⁷. The potential of the CO_2 laser to generate laser pulse energy with an efficiency of $\sim 10\%$, and its high-average-power capability for possible reactor applications, made it an attractive candidate. But the coupling of $10.6\ \mu\text{m}$ CO_2 laser light to dense target plasmas occurs at electron densities of $10^{19}\ \text{cm}^{-3}$, where electron-ion damping of plasma instabilities is weak, making the production of a very nonthermal plasma, unsatisfactory for inertial fusion applications, a distinct possibility. The attractiveness of the CO_2 laser system, and uncertainty over the seriousness of these plasma problems, led the group at the Los Alamos National Laboratory to develop CO_2 lasers having outputs of 10 TW (the Helios laser) and 30 TW (the Antares laser system) (ref. 9; see also similar

reports from the Los Alamos National Laboratory, 1971 to present).

At the Lawrence Livermore National Laboratory (LLNL), the Nd:glass laser was chosen as the major tool for research into the laser-plasma interaction¹⁰. Attractive features of the neodymium system are the ease with which pulse duration and pulse shape can be varied, the high optical-damage thresholds (which lead to moderately compact systems), the conveniently available beam diagnostics using film, silicon detectors or vidicons, and the scalability (through increased beam area or multiple beams) to high powers, which now exceed 100 TW and which can reach 1,000 TW. A variety of very efficient harmonic conversion techniques were also invented for this system, and these have been used to produce laser energy for a wide variety of experiments at 0.53 , 0.35 and $0.26\ \mu\text{m}$ (refs 11–13).

During the 1970s, experimenters used these systems and showed that both $10\text{-}\mu\text{m}$ and $1\text{-}\mu\text{m}$ laser wavelengths are too long for effective plasma heating. Studies using light at 0.5 , 0.35 and $0.26\ \mu\text{m}$ from harmonically converted Nd:glass lasers showed satisfactory plasma heating and confirmed earlier simulations. As a result, most ICF research is now being conducted with harmonically converted Nd:glass lasers. This research is being done in the United States at KMS Fusion Inc., Lawrence Livermore National Laboratory, the University of Rochester and the Naval Research Laboratory; in Europe at the Rutherford Laboratory and the Atomic Weapons Research Establishment, Aldermaston (both in England); at CEA Limeil in France; at the Lebedev and Kurchatov Institutes in Moscow; at the Max Planck Institute in Garching, FRG; and in Japan at Osaka University.

Research to provide other sources of short-wavelength light is also progressing. This work centres on the krypton fluoride laser¹⁴, with output at $0.249\ \mu\text{m}$, and the harmonically converted atomic-iodine laser^{7,15}, with outputs at 1.3 , 0.65 , 0.44 and $0.32\ \mu\text{m}$. These systems are being developed at several laboratories, but they have not yet been used for target experiments at multiterawatt levels.

While it is now clear that Nd:glass laser technology will suffice for the necessary laser-plasma research, it was thought necessary to invent a separate high-average-power, efficient laser technology for potential commercial applications. This process has been ongoing since the mid-1970s; there is not space here to review progress in this area. One surprising result of these studies, described in ref. 16, is the extent to which advanced solid-state systems, building on Nd:glass technology, may play a role.

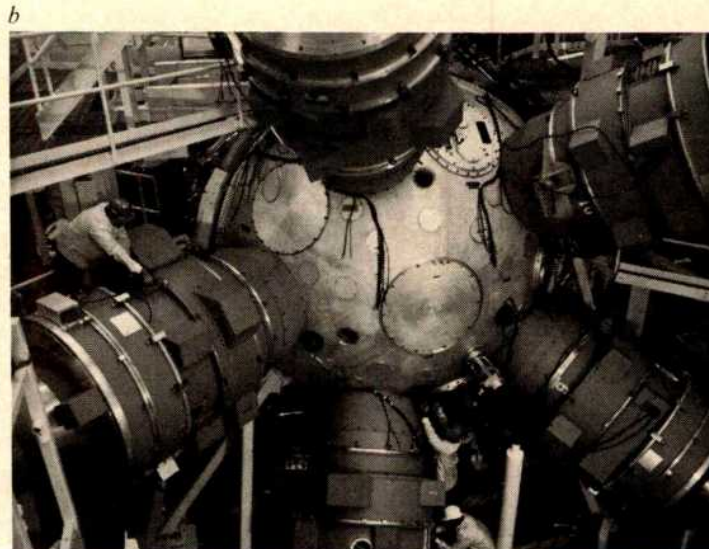


Fig. 2 *a*, The 10-beam Nova laser will provide 80–100 TW of peak power at $1.05\ \mu\text{m}$ and 40–70 TW of power at 0.53 or $0.35\ \mu\text{m}$ for fusion experiments. The pulse duration (and pulse shape) is variable from 70 ps to >10 ns. *b*, The target chamber. The large barrels attached to the target chamber contain the harmonic conversion arrays and the focusing lenses.

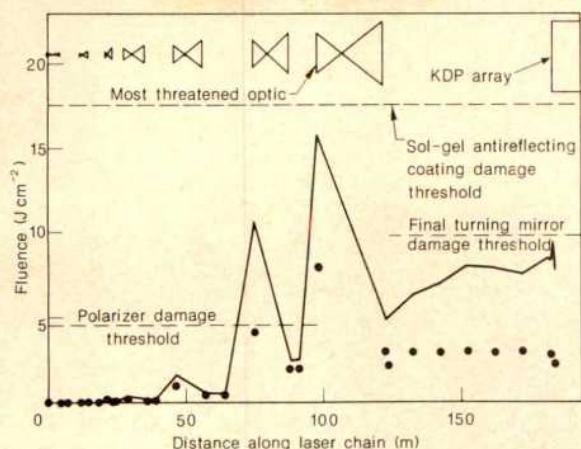


Fig. 3 The Nova laser beam is expanded, spatially filtered and re-imaged (projected) from a smaller amplifier section to the next larger section. Interchain peak (solid line) and average fluences (dots) as a function of distance along the laser chain, are shown for a 10.5-kJ laser pulse 1 ns long. The sizes and locations of the spatial filter lenses are indicated along the top of the figure. Entrance-lens surfaces will suffer damage if the peak fluence exceeds the indicated limits.

Laser physics and design

The physical constraints that control laser operation at 10 TW are reasonably well understood, and recently constructed systems have been designed to accommodate them¹⁷. These constraints are associated with the generation and shaping of the initial laser light pulse, the production and control of gain (and loss), the control of linear and nonlinear propagation along the beam path to keep the beam highly focusable, and the harmonic conversion of the high-power fundamental laser wavelength. Additional engineering concerns include cost, maintenance, and the diagnostics and control of the laser-target system.

The design of present Nd:glass lasers aims at realizing gain and propagation performance as close as possible to physical limits. While we use the Nova fusion laser as an example¹⁸, the design of high-power laser systems based on the other media is subject to similar constraints.

The amplification of a 10^{-4} -J, 1-mm-diameter oscillator pulse to a $\sim 10^4$ -J, 74-cm beam is accomplished by paying careful attention to the optical properties of the laser media and the optical system. Astronomical telescopes are used to enlarge and project the laser beam from the oscillator to the target-focus lens. The telescopes also project and image the beam shape from one stage to the next down the chain¹⁹; this prevents the beam shape from diffracting and developing highly modulated

diffraction 'ripples' as it propagates. That is, a well-shaped (apodized) beam that effectively fills the laser aperture can be magnified, projected through each laser amplifier where it is intensified, then further magnified and amplified in the next stage until it reaches the desired energy (consistent with the optical-damage threshold of the final element and with nonlinear self-focusing limits), and projected and amplified again (see Fig. 3). The telescope also serves a second important function, that of spatial filtering¹⁹. At the focus of the telescope system the laser beam is passed (in vacuum) through a pinhole that removes the high-frequency spatial noise from the beam. A second telescope lens then recollimates the expanding beam and projects a 'smoothed' image of the input aperture a distance Mf further downstream, where M is the magnification and f the length of the spatial filter. (A consequence of this approach is that the long distance travelled by the beam in Nova-like lasers is mostly through telescopes.)

The laser gain is determined by the stimulated-emission cross-section of the laser medium, by the pumping-rate limits set by the power supply, and by one of two gain-limiting processes. The first of these processes is stimulated depumping by amplified spontaneous emission (ASE) and the second is feedback oscillation (usually unexpected) from reflecting sources such as metal lens holders, screwheads, or the walls of the amplifier case. Stimulated depumping occurs when a spontaneously emitted photon traverses the gain medium and stimulates other ions to de-excite, thus depumping the medium. When the 'gain-coefficient $\times$ gain-length product (αl)' in the laser medium exceeds 4.5, this rate approaches 30% of the pumping rate for usual pumping situations. A greater loss rate than this is undesirable. Furthermore, at such a gain level, $G = \exp(4.5) = 90$, a 1.1% or larger reflection can cause a parasitic oscillation to start. This is avoided in glass lasers by cladding the edges with an index-matching absorbing glass yielding $<10^{-3}$ reflectivity. In the largest Nova amplifier (see Fig. 4), the laser plates are also cut and clad along the middle to control the transverse gain path and thus the ASE loss.

The efficiency of gain production is a complex function of the power-supply to gain-medium transfer efficiency (typically 1%) and the gain-medium to laser-light transfer efficiency (typically 25%). In research lasers, system efficiency is important only insofar as it (and the per-unit cost of power-supply) contributes to the total system cost. The very low cost of flashlamps and discharge capacitors compensates for the present low efficiency to provide acceptable power supply costs. However, for commercial applications such as the production of electricity or nuclear fuel, system efficiencies near 10% would be necessary²⁰.

Gain is developed when Nd^{3+} ions in optical glass plates are pumped by xenon flashlamps for 600 μs duration in an amplifier structure (Fig. 4). The gain coefficient α in the glass in the larger

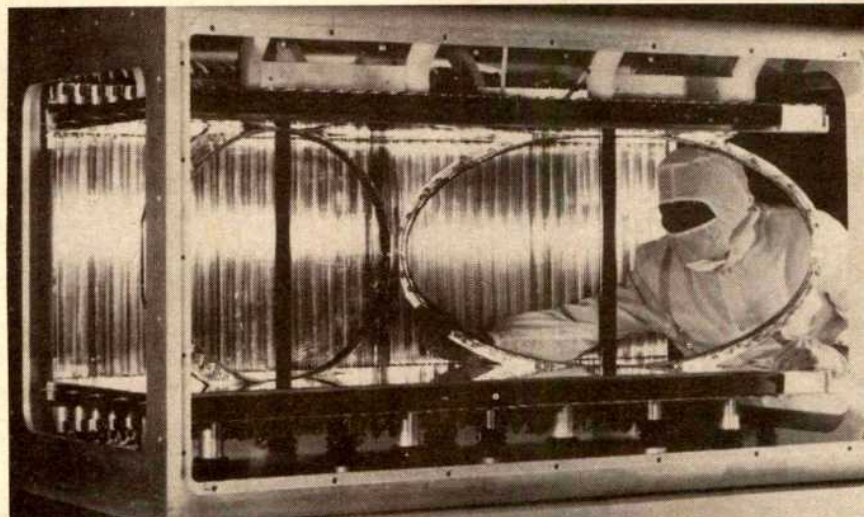


Fig. 4 The neodymium glass plates are mounted at Brewster's angle with respect to the laser beam to provide zero reflection loss. Light from the flashlamps excites the Nd^{3+} ions to produce gain.

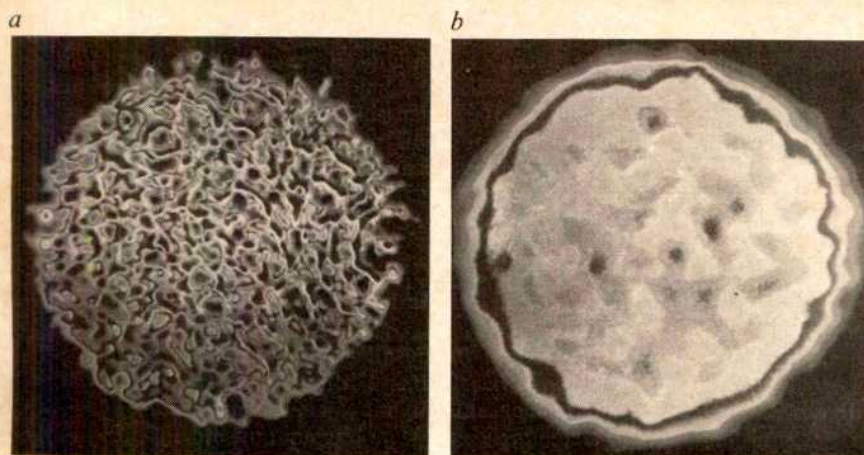


Fig. 5 High-power laser beams will self-focus when propagated at high powers through long glass paths. The criterion is given by the B -integral and by the spatial noise sources in the laser system. When spatially filtered, the beams are smoothed so that further propagation is possible. *a*, Into the spatial filter; *b*, exit from the spatial filter.

amplifier is typically 0.05 cm^{-1} , so that the beam must traverse a total path of 45 cm in the gain medium to reach a gain of 10. Ten 4-cm plates set at Brewster's angle in a series of disk amplifiers like those shown in Fig. 4 are used to provide the gain path in this amplifier stage. Six stages of amplification are used in each beam line to amplify the beam from 1 J at the entrance to 10^4 J at the exit.

As the beam propagates down the laser chain, its phase front is modified by linear and nonlinear phase aberrations. Linear aberrations cause small modifications to the phase front that reduce the focusability of the beam on the target. The optical glass in which the neodymium ions are dissolved can be made homogeneous to 2 parts in 10^6 , which would yield approximately 2 waves of aberration per metre of path in glass. Present polishing techniques are capable of compensating for these aberrations: when the beam passes through the 150 surfaces and 3 m of glass in a Nova laser beam, an acceptable 2–3 waves of total linear aberration result.

The most nonlinear (and permanent) impediment to beam propagation is optical damage. The energy fluence in the beam, $5\text{--}10 \text{ J cm}^{-2}$, is enough to cause the explosion of small absorbing inclusions in coatings or within laser glass or lenses, obscuring part of the beam and permanently damaging the laser component. Laser glass and other transmitting optics can be made inclusion-free², which will allow them to sustain intensities greater than $10^{10} \text{ W cm}^{-2}$ in 1-ns pulses. However, to obtain good production yields of inclusion-free glass for reliable operation at these fluence levels, additional work is required. A great deal of effort has been expended on developing coatings that can withstand operation at high fluence. Particularly successful anti-reflection coatings based on 'sol-gel' coating technology and on 'neutral-solution' surface etching processes have been produced and used in the Nova laser²¹. These coatings are virtually defect-free and exhibit damage thresholds three times higher than those produced using electron-beam deposition procedures.

A more gradual nonlinear beam limit is caused by self-focusing¹⁷. At intensities $>10^9 \text{ W cm}^{-2}$, the electric field of the laser beam exceeds 10^6 V cm^{-1} . This field is strong enough to modify the electron orbits around the atoms or molecules in the medium through which the beam propagates, changing the index of refraction. This causes a self-focusing instability to grow at a rate proportional to $\exp(2B)$, where B is the so-called breakup or B -integral

$$B = 0.026 \frac{n_2}{n\lambda} \int I \, dl \quad (1)$$

Here n and n_2 are, respectively, the linear and nonlinear indices of refraction in electrostatic units, λ is the laser wavelength in μm , I is the intensity in GW cm^{-2} , and dl is the element of path length in cm. The breakup integral must have a value less than ~ 3 for propagation to occur without severe loss. The nonlinear index of refraction changes the index of refraction by $\sim$ one

part in 10^6 at peak intensity. This change is small (it corresponds to less than one wave of beam phase retardation per metre of glass path traversed). But if the transverse dimension over which it takes place is small ($\sim 1 \text{ mm}$), the induced focal power of the resulting 1-mm-diameter 'lenslet' will cause a 1-mm portion of the beam to self-focus, and the corresponding energy will not be available for harmonic conversion or for focusing on a target (see Fig. 5). Nonlinear beam self-focusing was the chief cause of nonreproducibility in early laser-target experiments. In extreme cases, the entire laser beam self-focused into thousands of small light filaments that could not be focused on the target and which 'drilled' small holes in the laser glass, the mirror coatings and the lens surfaces. Control of nonlinear self-focusing made Nd:glass lasers useful for target irradiation and made them scalable to much higher power than was at first predicted.

To provide adequate gain and to control self-focusing, neodymium-doped phosphate glass was selected as the gain medium. Its gain characteristics are compatible with 5-J cm^{-2} output fluence, and its self-focusing characteristics permit the propagation of twice as much optical fluence as in silicate laser glasses. This permitted propagation at higher fluences through longer gain-medium paths than in previous fusion lasers, such as the Shiva laser at LLNL. The longer gain path is needed to amplify the laser pulse to the 5-J cm^{-2} (5-GW cm^{-2}) level at 1-ns pulse duration, or to 10 J cm^{-2} when the system is used for experiments with pulse durations $>3 \text{ ns}$. The lower nonlinear index of refraction of the phosphate glass permits high-power operation with very short pulses ($\sim 0.1 \text{ ns}$) also.

Harmonic conversion

Harmonic conversion is used to generate shorter wavelengths for target irradiation. This is feasible because of the high phase uniformity of high-power beams from modern lasers, and because the power density in such beams is high enough (GW cm^{-2}) to drive a nonlinear polarization in the atoms or molecules of non-centrosymmetric crystals. The magnitude of the linear polarization is

$$P = \chi \epsilon_0 E = 10^{-4} \text{ debye per molecule per GW cm}^{-2} \quad (2)$$

where χ is the susceptibility, ϵ_0 is the vacuum permittivity, and E is the electric field of the light wave. If a non-centrosymmetric crystal is used, the restoring force on the electron cloud is not symmetric about the equilibrium position, and the susceptibility can become proportional to E :

$$\chi = \chi_0 - \chi_1 E + \dots \quad (3)$$

When equation (3) is substituted into equation (2), there results a term in the polarization, $\chi_1 \epsilon_0 E^2$, that leads to an oscillating dipole at twice the fundamental frequency ω_0 of the electric field. A typical $2\omega_0$ nonlinear polarization is 10^{-9} debye per molecule, or 10^{-5} times the linear polarization.

One can imagine this nonlinear polarization developing in the first plane of atoms in the crystal as the beam strikes the

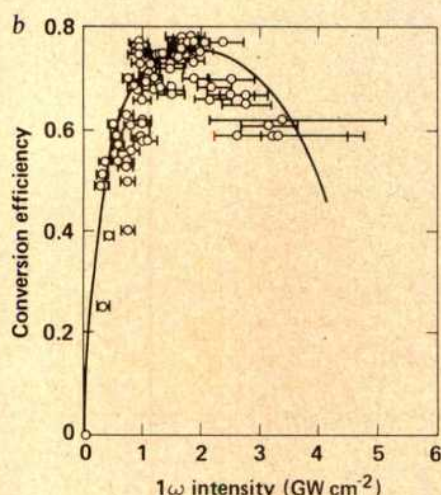
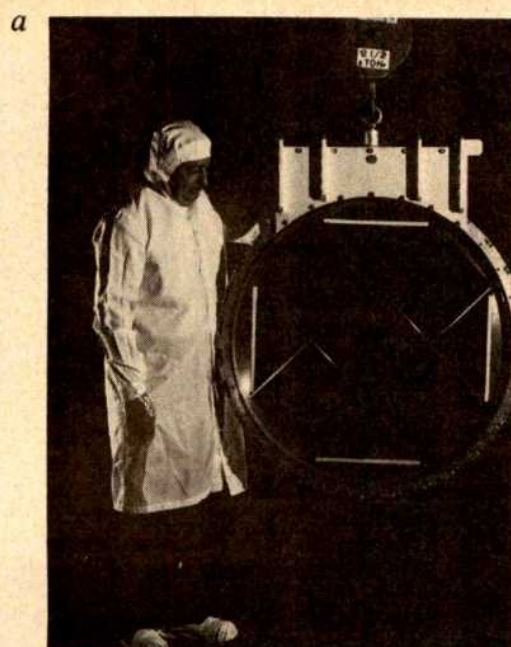


Fig. 6 High-power, 1.05- μm laser beams are sufficiently uniform in phase to uniformly convert to 0.53, 0.35 or 0.26 μm with high efficiency. *a*, A 3×3 array of 1.8-cm-thick KDP harmonic conversion crystals. *b*, Conversion efficiencies with a similar array on the Novette laser.

surface. We can arrange for the radiated waves from each plane of atoms to add coherently to those of the succeeding planes, so as to generate a strong harmonic wave; we do this by choosing the propagation direction in uniaxial or biaxial crystals so that the phase velocities of the harmonic and fundamental waves are the same (the waves are then said to be phase-matched). Phase-matching allows for very efficient harmonic conversion when the phase front of the incoming fundamental laser light is uniform enough to maintain these conditions over the entire beam area and throughout the length of the crystal. For example, with a properly oriented 1.8-cm-thick crystal of potassium dihydrogen phosphate (KDP), we can convert 1.05- μm laser light at 2 GW cm^{-2} to the second harmonic at 0.53 μm with up to 80% efficiency¹¹⁻¹³. When losses in the optical components (lenses, diagnostics, blast shields) of a fusion laser beam line are taken into account, it is possible to deliver over 50% of the laser's 1.05- μm energy to a target at 0.53 μm ; 70% conversion efficiency may be expected with additional engineering improvements^{22,23} (Fig. 6).

By using a second crystal after the first crystal, efficient conversion to the third harmonic at 0.35 μm or the fourth harmonic at 0.26 μm is possible. For the third-harmonic process, the

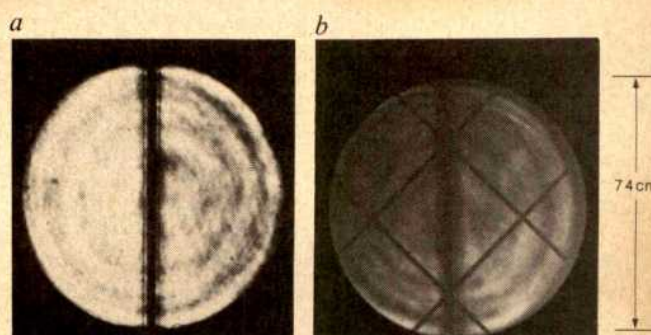


Fig. 7 Amplitude pictures of 74-cm-diameter beams on Novette. The quality of these beams permits focusing to 100- μm -diameter focal spots, leading to power densities $>10^{17} \text{ W cm}^{-2}$ on target. However, most target irradiation is conducted at 10^{14} – $10^{15} \text{ W cm}^{-2}$. The dark vertical line in the middle of the beam is due to the gain-parasitic absorber in the largest amplifier. The 3×3 checkered pattern is due to the array of harmonic conversion crystals. *a*, 10 TW, 1.05 μm ; *b*, TW, 0.53 μm .

second crystal is oriented to phase-match a one-to-one photon mix of two input waves, one at 0.53 and the other at 1.05 μm , which beat together through the χ_1 term in equation (3) to produce the output wave at 0.35 μm . Experiments at Rochester and at Lawrence Livermore have demonstrated third-harmonic conversion efficiencies of up to 80% (refs 11–13). For the fourth-harmonic process, the second crystal is oriented to convert a 0.53- μm wave from the first crystal to 0.26 μm .

The Nova laser

The Nova laser was designed to be a versatile experimental system capable of irradiating targets with pulse durations from 0.05 ns to over 10 ns at wavelengths of 1.05, 0.53 and 0.35 μm (ref. 24). Experiments at 0.25 μm can be accomplished with some modification. In 1982, early production of two of Nova's 10 beams were configured into a facility called Novette^{22,23}, which was used to verify the Nova laser design and the process of harmonic conversion to 0.53 μm , and to verify models of plasma heating by 0.53- and 0.25- μm laser light. This work was largely successful²⁵. The objective of the full 10-beam Nova laser is to extend these results and produce conditions of DT-fuel compression and laser-plasma coupling similar to those required by high-gain targets.

The diameter of Nova's beams posed special problems for harmonic conversion. Our solution was to assemble slices cut from 27-cm-wide KDP crystals into a 3×3 mosaic array with a 74-cm-diameter aperture (see Fig. 6). A significant technological innovation was the use of a diamond cutting tool on a precision lathe to cut each crystal surface to the required smoothness and flatness and to the correct angle with respect to the crystal axes. In addition, it was necessary to develop coatings, mechanical supports and diagnostic techniques. The final harmonic system is remarkably simple, using advanced sol-gel coatings and a two-crystal design that efficiently generates 0.53 μm or 0.35 μm light with a simple tilt ($1/4^\circ$) and rotation (10°) of the array. A singlet-lens focusing system, using chromatic dispersion in the lens, together with a central beam block, can focus the beam at the fundamental 1.05- μm wavelength, or at the two harmonic wavelengths, without other, unwanted wavelengths reaching the target.

At the highest beam powers delivered by Nova, stimulated nonlinear optical processes can occur: stimulated Raman scattering from nitrogen in the beam path, additional nonlinear self-focusing in the air path, and transverse stimulated scattering in Raman-active materials (for example, the KDP crystals) were the expected possibilities. Careful measurements of the thresholds for these processes gave us confidence that the system would perform satisfactorily at the desired powers. With the Nova beams finished, we have now measured a gain of 2.7 cm per TW for stimulated Raman scattering in a very long beam

path²⁶. This gain is high enough to limit Nova's output power, so we are planning to remove the nitrogen from the beam tube. Nova's output in 1-ns pulses will be 80–100 kJ at 1.05 μm , with better than 50% conversion to 0.53 or 0.35 μm , depending on the pulse duration. Figure 7a, b shows the 1.05- and 0.53- μm beam images, respectively, from a Nova beam when it was used on the Novette laser.

Future developments

The future development of high-power solid-state lasers will follow two directions. A 5–10-MJ, 1,000-TW laser will be required to demonstrate and optimize the performance of high-gain ICF targets (see Fig. 1). A high premium will be placed on the versatility and cost of this system technology, and on the utility of the technology in future industrial, defense and ICF applications. The second direction is toward high average power and very efficient operation at moderate cost. Systems with these characteristics would be required for electrical power generation or for production of nuclear fuel.

Work on both types of systems is progressing. Considering our views in the early 1970s, we are surprised that the scale of the Nd: glass laser, as well as those of the I, KrF and CO₂ lasers, can indeed be increased so as to reach the energy and power

levels necessary for high-gain fusion demonstrations. The harmonically converted glass laser appears to offer the most flexible and economical approach to meeting this objective. This conclusion is based on the physics constraints of the competing systems and on the engineering and manufacturing experience developed in building large CO₂ and glass fusion-laser systems and smaller experimental KrF laser systems. The prospect of high-repetition-rate (5–10 Hz), highly efficient (>10%) solid-state lasers using crystalline hosts rather than glass hosts and using solid-state pumping rather than flashlamp pumping has been theoretically supported by Emmett *et al.*¹⁰ and is in the process of experimental demonstration.

I thank my colleagues in the Laser Fusion Program at the Lawrence Livermore National Laboratory who have worked to design, construct and use these systems. In particular, J. L. Emmett has been responsible for the overall programme direction. W. F. Krupke developed many advanced laser concepts, J. B. Trenholme, W. F. Hagen and W. E. Warren developed much of our design methodology, J. T. Hunt and D. R. Speck are readying Nova for target experiments, R. O. Godwin and W. W. Simmons designed and managed Nova construction, K. R. Manes and G. J. Suski led the team that built and used Novette, the precursor to Nova, and D. Eimerl and M. A. Summers provided many of the harmonic conversion ideas.

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Laboratory production of X-ray lasers

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Laser-produced plasmas seem to be the most promising sources of soft X rays at the high intensities required for laser action. Strong laser amplification has recently been demonstrated at the lowest X-ray energies, suggesting that true soft X-ray lasers will soon be available in the laboratory.

A MAJOR objective in laser research is to develop X-ray lasers¹. Optical lasers revolutionized optical methods because of the high power, collimation, spectral purity and coherence of their beams. Similar, dramatic consequences could be expected if X-ray lasers became widely available.

Many important scientific applications of intense X-ray emission are already being developed using synchrotron radiation sources². These emit incoherent X-rays from circulating bunches of highly energetic (GeV) electrons deflected by the magnetic field in an accelerator storage ring. A pure continuum of frequencies from infrared to X-ray is generated with high average power in subnanosecond pulses at high (100 MHz) frequency. Monochromators are used to select particular frequencies for applications such as diffraction or spectroscopy, and the emission pulses are accumulated over periods from seconds to hours to obtain sufficient signal.

More recently, single-pulse X-ray measurements have become possible using laser-produced plasmas³. Their incoherent emission results from thermal collisions of electrons with ions in a hot plasma produced by focusing a powerful laser pulse on to a solid target. A continuum of frequencies is emitted by free electrons and narrow lines are emitted by electrons bound to ions. The single-pulse spectral brightness is up to 10⁶ times greater than for synchrotron radiation sources as illustrated in Fig. 1.

To produce coherent laser X-rays has proved more difficult. For a constant laser amplification coefficient, the required fluorescent emission per unit volume on the laser transition scales as frequency $\nu^{4.5}$. Hence there is a need for exceptionally intense X-ray sources for X-ray lasers, the most promising of which seem to be laser-produced plasmas. In this context, a recent experiment at the USA Lawrence Livermore National

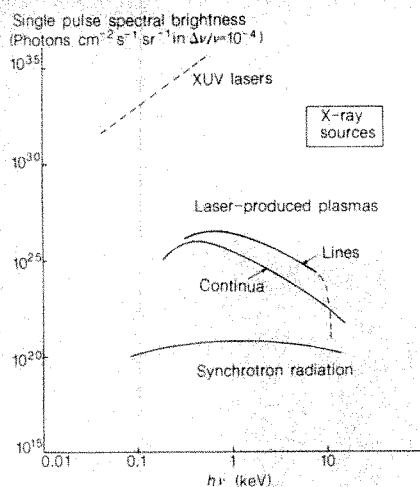


Fig. 1 Comparison of the absolute spectral brightness of XUV and X-ray sources. (Synchrotron data are from the SERC Synchrotron Radiation Source (SRS) and laser-produced plasma data are compiled for 10^{12} watt Nd glass lasers.)

Laboratory (LLNL) has generated much excitement by convincingly demonstrating strong laser amplification at a wavelength of 20.6 nm (photon energy $h\nu = 60$ eV; where h is Planck's constant) in a laser-produced plasma⁴. This is the most significant recent advance in short-wavelength lasers, the previous limit having been set at 116 nm ($h\nu = 10.7$ eV) in 1972 with the discovery of the Werner band H_2 laser. Although 116 nm has been the limit for laser action, generation of the seventh harmonic of ultraviolet laser light in gases has extended the short wavelength limit for high-power coherent radiation to 30 nm⁵.

It should be emphasized, however, that 60 eV is only an extreme (X) UV photon energy; a true soft X-ray laser will require a further order of magnitude increase in photon energy. However, the significance of the recent progress at LLNL is that it gives more confidence that a new family of XUV lasers based on high temperature laser-produced plasmas can be developed in the XUV/soft X-ray region >60 eV. Here I describe the characteristics of these lasers, explain how they operate, review recent progress and speculate on their future applications.

Background

All lasers have in common the fact that they produce light amplification by stimulated emission of radiation, hence their name. Stimulated emission was first postulated by Einstein as the quantum inverse of absorption. A quantum of electromagnetic wave energy (or photon), whose energy is $h\nu$, can resonantly stimulate the emission of an identical photon by an atomic electron in an excited state if the energy of the excited state E is $h\nu$, greater than that of a lower state. The new photon is coherent with the stimulating photon, that is the electromagnetic waves have the same phase. Stimulated emission amplifies electromagnetic waves and the inverse process of absorption attenuates them.

The only difference between an XUV laser and a more usual visible light laser is that the laser transition is an XUV frequency. Such transitions occur in high temperature plasmas of highly charged ions and electrons. The reason can be seen in Bohr's elementary model of a hydrogen-like ion illustrated in Fig. 2. The positive nucleus, with one orbiting electron, has a system of energy levels in which the transition energies are

$$h\nu = Z^2 h\nu_H \quad (1)$$

where Z is the charge number of the nucleus and $h\nu_H$ is the transition energy in a hydrogen atom ($Z = 1$).

For example, the Balmer α transition illustrated in Fig. 2 emits XUV photons ($h\nu \sim 68$ eV) when $Z = 6$. The hydrogen-like ion is produced by removing five electrons from a carbon atom leaving a positive nucleus with a single electron, which is isoelectronic (having the same number of electrons) as a hydrogen

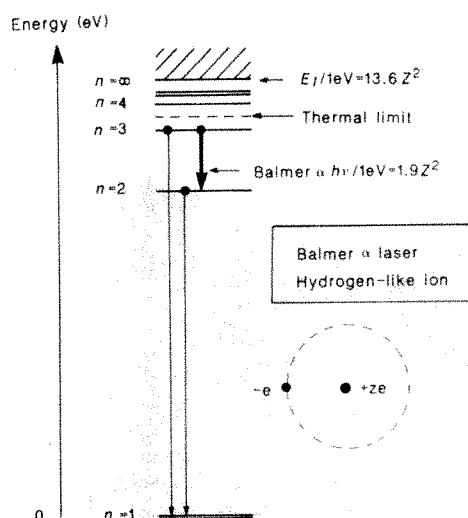


Fig. 2 Energy levels of a hydrogen-like ion of nuclear charge $+Ze$ showing the location of the thermal limit when population inversion is obtained between $n = 2$ and $n = 3$.

atom. Such isoelectronic scaling to higher Z shifts the emitted frequency towards the X-ray region.

Production of highly charged ions in plasmas is described by a well-known equation due to Saha and requires high temperatures such that the energy kT in collisions between electrons and ions is an approximately constant fraction f of the ionization energy $Z^2 E_H$

$$kT = fZ^2 E_H \quad (2)$$

The net amplification coefficient resulting from the balance between absorption and stimulated emission on any atomic transitions can be expressed as

$$G = (n_2 - n_1(g_2/g_1))(\lambda^2/8\pi)(A_{21}/\Delta\nu) \quad (3)$$

where n_1 and n_2 are number densities of the lower and upper states of degeneracy g_1 and g_2 , respectively, and A_{21} is the Einstein A coefficient or probability per unit time of a spontaneous transition from the upper to lower state of wavelength λ .

The spectral width $\Delta\nu$ of the transition due to the Doppler shift arising from the thermal velocity of the ions scales as

$$\Delta\nu/\nu \propto (kT)^{1/2} \text{ implying } \Delta\nu \propto \nu^{3/2} \quad (4)$$

There is also a fundamental frequency scaling of the Einstein A coefficient in an isoelectronic sequence

$$A_{21} \propto \nu^2 \propto \lambda^{-2} \quad (5)$$

Amplification (that is, negative absorption) is obtained with population inversion (excess of excited states), that is,

$$n_1(g_2/g_1) < n_2 \quad (6)$$

Equations (3) and (5) show that to sustain a given amplification requires a minimum density of excited states

$$n_2 \propto \Delta\nu \quad (7)$$

The spontaneous fluorescent emission from these excited states has a power per unit volume,

$$p = n_2 A_{21} h\nu \quad (8)$$

and it follows from equations (4) to (8) that

$$p \propto \nu^3 \Delta\nu \propto \nu^{9/2} \quad (9)$$

and it is this $\nu^{9/2}$ scaling of the fluorescent power that makes it very difficult to achieve laser action at high frequencies.

Another major problem is to find methods of creating population inversion when nature tends to create thermal equilibrium in which the well-known Boltzmann relations apply, giving no amplification at any temperature, that is

$$n_2(g_1/g_2) = \exp(-h\nu/kT)n_1 \quad (10)$$

Thermal equilibrium is a consequence of thermodynamic detailed balance in which each transition inducing process is balanced by its inverse process, for example



here collisional ionizations are balanced with three-body recombination. Collisional processes are always balanced by their inverse and therefore in the limit of high plasma density, where collisions are dominant, thermal equilibrium level populations prevail. But spontaneous radiative transitions are most rapid across large energy gaps ($A_{21} \propto \nu^2 \propto Z^4$), whereas for collision induced transitions (of rate $K_{21}n_i n_e$, where n_e and n_i are the number densities of electrons and ions) the opposite is true, with the rate coefficient K_{21} scaling⁶ as

$$K_{21} \propto \nu^{-1} kT^{-1/2} \propto \nu^{-3/2} \propto Z^{-3} \quad (12)$$

The result is that we can identify, in a plasma of lower density, a 'thermal limit' as illustrated in Fig. 2. Above the limit, where energy levels are closely spaced, collisions dominate and below the limit radiative rates are greater than collisional rates. Radiative processes are not in detailed balance unless spontaneously emitted photons are reabsorbed by the plasma. This will not occur unless the plasma volume is large. Thus levels below the thermal limit need not be in thermal equilibrium and, with suitable tricks, population inversion can be created, for example, between levels of principal quantum number $n = 3$ and $n = 2$ in Fig. 2.

In isoelectronic scaling of laser mechanisms to shorter wavelengths it is generally necessary to preserve the balance between radiative and collisional processes so that the thermal limit remains at the same principal quantum number. From the preceding discussion it follows that the temperature $kT \propto Z^2$ and the particle number density $N \propto Z^7$ requiring plasmas of progressively higher temperature and density for lasers of higher frequency.

XUV laser characteristics

The new family of XUV lasers will probably be amplified spontaneous emission (ASE) lasers using single or double transit amplification of spontaneous emission in a long thin volume of laser medium as illustrated in Fig. 3. They are unlikely to have the usual two mirror laser resonator because of the combined problems of short duration of amplification, mirror damage, difficulty of output coupling and criticality of alignment. Here I outline the performance characteristics of such lasers.

The single transit amplification is $\exp(GL)$, where G is the amplification coefficient and L the length. The ASE laser requires a huge single transit amplification to reach the saturation condition where stimulated emission becomes the dominant process. Any plasma-based laser would require $GL \geq 14$, that is, $\exp(GL) \geq 10^6$.

The angular divergence of the laser beam is simply the width to length ratio w/L of the gain medium for a single transit (or the potentially much smaller width to overall length ratio w/L_0 for a double transit with a single mirror), as shown in Fig. 3 and will typically be 10^{-3} – 10^{-2} rad. Further simple atomic physics and plasma physics arguments show that the saturated output power of the laser depends mainly on the frequency and is proportional to the beam area. Assuming a beam area of 10^{-4} cm², typical of the cross-sectional area of the plasma (Fig. 3c) the output power is in the megawatt range

$$P = (h\nu/100 \text{ eV})^{4.5} \text{ MW} \quad (13)$$

The duration of the output pulse depends on the laser mechanisms discussed below and will probably be in the range 50 ps to 1 ns for the new family of ASE lasers.

The fluorescent spectral line width of the laser is usually determined by Doppler broadening giving $\Delta\nu/\nu \sim 10^{-4}$, but laser spectral narrowing further reduces the line width in the laser beam by a factor of ~ 4 . The spectral brightness for the previously discussed parameters is plotted in Fig. 1, showing the dramatic increase relative to even laser produced plasma sources.

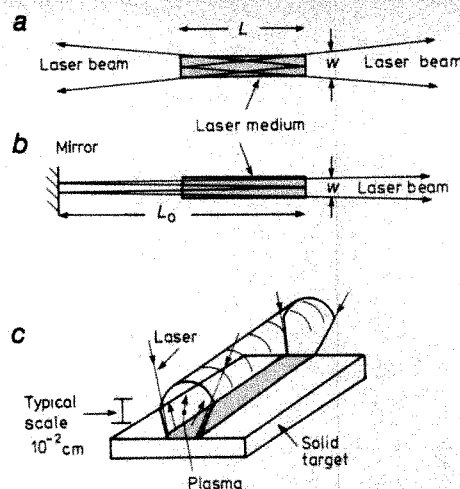


Fig. 3 Schematic single-pass (a) and double-pass (b) ASE lasers. c, Schematic model of plasma production by a laser beam focused on to a line.

Emission from an ASE laser is coherent in time only for a period of the order of the inverse spectral bandwidth $\Delta\nu^{-1}$ which from the earlier discussion means $\sim 4 \times 10^4$ cycles of the electromagnetic wave. The spatial coherence across the wavefront is determined by the rather large number ($n = w^2/L\lambda$) of transverse modes excited within the laser beam angle w/L . Transverse spatial coherence is restricted therefore to regions of dimensions of about $1/n$ (typically $1/50$) of the beam diameter. The use of a single mirror to double pass the laser medium offers improvements in spatial coherence through reducing the number of transverse modes.

Experiments and laser schemes

Proving population inversion and amplification. High-power pulsed lasers focused on to solid targets produce small hot expanding plasmas with dimensions 0.1 – 0.2 mm³. The existence of population inversion on XUV transitions was first determined and quantified in such plasmas from the ratio of intensities of spectral lines originating from the potential laser levels (for example, in Fig. 2 the population ratio n_3/n_2 is given by the intensity ratio I_{31}/I_{21} and the absolute intensity I_{31} gives the absolute number density of excited states n_3). Measurements with resolution in space and time were developed for greater accuracy⁹.

Demonstration of population inversion did not prove the presence of net amplification because of the possibility of competing absorption processes such as the photoionization of other ions in the plasmas. In the next phase of experiments direct evidence of amplification was sought using elongated plasmas produced most often in a line focus of length $L = 1$ or 2 mm (this was limited by the available laser power) as illustrated in Fig. 3(c). GL values were measured from the ratio $R(L)$ of spectral line intensities for the laser transition along the axis and transverse to the axis of the plasma. This ratio is theoretically $(\exp(GL) - 1)/GL$ ¹⁰. Earlier experiments were generally not very accurate because GL values were small.

A few very recent experiments have used much longer line foci with $L = 1$ – 2 cm, and new time-resolving XUV spectrometers. With larger GL , variation of the length L between, say L_1 and L_2 allows a measurement of the ratio $I(L_1)/I(L_2)$ which is theoretically $(1 - \exp(GL_1))/(1 - \exp(GL_2))$. Direct evidence of exponential amplification has been obtained in this way. In particular the recent experiments at the LLNL showed GL values ≤ 6.5 (ref. 4).

3p–3s transitions in neon-like ions. The LLNL experiment used an idea which is derived from the mechanism of the familiar argon ion laser and was first suggested in the USSR¹¹. Other researchers followed up the concept^{12,13}, but most effort was confined to the USSR¹⁴ until a few years ago when, as part of

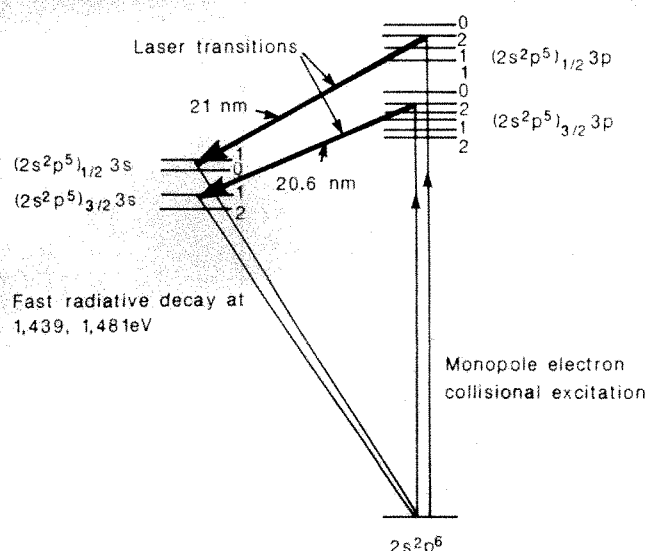


Fig. 4 Energy-level structure of Se^{24+} , after D. L. L. Matthews (personal communication).

a major campaign to test XUV laser options, the LLNL team began serious theoretical studies and experimental preparation for large-scale experiments using the two most powerful laser beams in the world from their new neodymium glass laser Novette.

The energy levels of the neon-like ions are illustrated in Fig. 4; the laser relies upon the fact that the ion has a closed shell of 2p electrons from which excitation by collision with plasma electrons populates the 3p states (as well as 3s and 3d states). There is no quantum mechanically allowed radiative decay from 3p to 2p, but there are strongly allowed transitions from 3p to 3s and 3s to 2p. If the thermal limit lies above the 3s level, then 3s to 2p radiative decay to the ground state reduces the 3s population and creates 3p to 3s population inversion.

The neon-like ion of selenium (Se^{24+}) has 3p to 3s transition energies of ~ 60 eV and was chosen for the LLNL experiments. A very thin (75 nm) layer of selenium was used to avoid reabsorption of 3s to 2p resonance radiation. The final important choice was to use a thin polymer film (50 nm) to support the selenium layer so that, when the target was irradiated with a 450 ps pulse at $5 \times 10^{13} \text{ W cm}^{-2}$, the whole target thickness was uniformly heated producing the Se^{24+} ions at a temperature of $\sim 5 \times 10^6$ K and causing an explosive expansion to a fairly uniform lower density (10^{21} electrons cm^{-3} or $3 \times 10^{-3} \text{ g cm}^{-3}$), at which maximum amplification occurred. The uniform density is vital to reduce refraction of the XUV laser beam as it travels along the 200 nm thick expanded layer of selenium plasma. Detailed calculations¹⁵ suggested amplification coefficients as high as $G = 10 \text{ cm}^{-1}$.

The two large (50 cm) beams of the neodymium glass laser were focused to a 12 mm $\times$ 200 μm line focus allowing up to 2 cm length to be irradiated. Sophisticated time resolving XUV spectrometers viewed the emission axially and transversely and Fig. 5 shows the clinching results. Two of the 3p to 3s lines at 20.6 and 21.0 nm were amplified by up to 500 times along the axis, indicating $G = 5.5 \text{ cm}^{-1}$. The output power was $> 100 \text{ W}$ with a divergence of 10^{-2} rad in a 300 ps pulse. A thermal source would need a temperature of $> 400,000,000 \text{ K}$ for this brightness. Despite the extreme brightness this is still only $\sim 1/3,000$ of the saturated output power which could be obtained with a modest twofold increase in GL . The importance of this result is considerable because it is the first absolutely incontrovertible evidence of high XUV gain. Limited isoelectronic scaling possibilities were also shown in the LLNL work with evidence of amplification in neon-like Yttrium at shorter wavelengths $\sim 15 \text{ nm}$.

Recombination. Population inversions are readily created in rapidly recombining plasmas, though this is not a mechanism

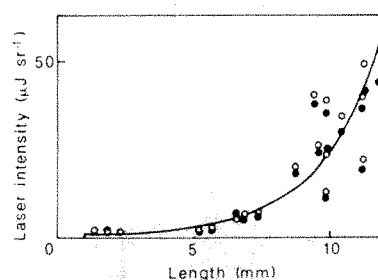


Fig. 5 Exponentially increasing output as a function of length demonstrating single-pass laser amplification of spontaneous emission in the Se^{24+} laser beam (redrawn from ref. 4). Solid circles, 209 Å; open circles, 206 Å; $-g = 5 \text{ cm}^{-1}$.

in any common type of laser. The idea, again Russian in origin¹⁶, dates from 1965 but isoelectronic scaling to the XUV was considered in the United States and the United Kingdom in the early 1970s (refs 12, 17, 18). Recombination from a plasma of bare nuclei to form hydrogenic ions, at densities such that the thermal limit is at $n=3$ or higher, can result in inversion of population between $n=3$ and 2, because of rapid radiative decay depopulating the $n=2$ level. This occurs only if the ground state has an anomalously low population, a situation which can be produced by sufficiently rapid cooling such that the relatively slow process of recombination to the ground state lags behind the fast collisional processes across the small energy gaps populating levels above $n=2$. Fortunately, such rapid cooling is produced almost automatically when a laser-produced plasma expands adiabatically into a vacuum from an irradiated target⁸.

Carbon plasmas have been extensively studied. Early evidence showed population inversion on the 18.2 nm $n=3 \rightarrow n=2$ transition at low densities^{8,9}. Subsequent attempts to produce laser action used cylindrical expansion from 1 to 2 mm long laser-heated carbon fibres. The fibre size was restricted to a few microns in diameter so that the mass of the expanding plasma was small enough to avoid reabsorption of resonance radiation on the 2 to 1 transition. The material of the fibre is heated initially to $kT = 500 \text{ eV}$, at a density $n_e = 10^{21} \text{ cm}^{-3}$. Heating occurs during a 200 ps laser pulse through absorption of between 2 and 20 Joules per cm length of the fibre. Rapid expansion and cooling in the following nanosecond reduces the density to 10^{19} cm^{-3} and the temperature to 20 eV. Recombination into the ground state is minimal. Detailed calculations indicate amplification coefficients from 1 to 5 cm^{-1} of duration $< 1 \text{ ns}$ ¹⁹. The lower amplification is produced paradoxically by higher absorbed energy in a non-critical mode of operation involving total burn through of the fibre. High amplification is obtained with lower mass plasmas produced by partial burn through of the fibre, but demands very precise conditions which are difficult to produce uniformly along by the length.

Experiments have shown evidence of GL up to 4, deduced from axial to transverse intensity ratios, but with poor reproducibility in the high gain mode¹⁰. Experiments in the stable low-gain mode have so far had insufficient plasma length for direct measurement of amplification ($< 2 \text{ mm}$) but have shown good agreement between measurements of the plasma hydrodynamic characteristics and computer modelling¹⁹.

A rather different approach to this kind of laser has been used in other experiments²⁰. Plasma expanding from a carbon target irradiated by a 500 J, 10 GW pulse from a CO_2 laser has been confined to a narrow 1 mm diameter cylinder by an axial magnetic field and rapid cooling has been caused by thermal radiation. Evidence from axial to transverse intensity ratios suggests GL values of the order of 6. Variation of the plasma length is not possible and the feasibility of making a useful laser is difficult to determine. An amplification coefficient of 3 cm^{-1} has also been reported for thin polymer film targets similar to those used in the Se^{24+} laser and radiation cooling from the Se is thought to compensate for the reduced adiabatic cooling of a planar expansion²¹.

Recombination of helium-like (2-electron) ions to the lithium-like (3-electron) configuration is more complex to analyse but is the basis of an almost accidentally discovered mechanism for XUV laser action at 11 nm which has shown quite significant amplification ($G \sim 3 \text{ cm}^{-1}$)^{22,23}. The best results were obtained using 3 GW, 20 ns Nd glass laser pulses and an 8 mm line focus on solid aluminium targets. The laser transition is the 5f-to-3d transition of lithium-like Al^{10+} . The GL value has been determined from the intensity ratio for different plasma lengths as discussed earlier. Measurements have also included time-resolved spectroscopy showing the duration of the gain to be a few ns. Isoelectronic scaling has been demonstrated in similar experiments with magnesium. There are problems however with competing absorption processes which are not well understood. The recombination lasers have amplification coefficient comparable to the 3p-3s lasers and are more attractive for scaling to short wavelength because they use transitions with a change of principal quantum number with strong Z^2 scaling of the transition energy in isoelectronic sequences. They suffer less from refraction because the plasma density is much lower (10^{19} cm^{-3} compared with 10^{21} cm^{-3}) allowing longer plasmas and larger GL values.

Resonant photopumping. Selective excitation by photons is used in many optically pumped lasers and the method can in principle be extended into the XUV as first suggested by Russian authors in 1975 (ref. 24) and elaborated on in subsequent more detailed studies^{25,26}. Current thinking centres on using intense resonance line emission from one species of ion in a hot laser-produced plasma, with a brightness at the line centre corresponding to a black body with $kT_H \sim 200 \text{ eV}$, to resonantly excite another ion species in an adjacent plasma, for example, the $1s^2$ - $1s2p$ resonance line of Na^{9+} at 1.1 nm is absorbed by the $1s^2$ - $1s4p$ transition of Ne^{8+} . If the second plasma has a low temperature, $kT_e \ll kT_H$, and a density such that the thermal limit is above the laser levels, the $n=4$ level may be populated to produce population inversions, such as in the above example for the $4 \rightarrow 2$ transition at 5.8 nm.

Predicted amplification is as high as $G = 100 \text{ cm}^{-1}$ (ref. 26) and potential laser wavelength can be quite short as in the example given. Many such resonances have been identified in H, He, Li and F-like ions²⁵ but as yet there has been no success in demonstrating gain experimentally. But experimental work is at an early stage and the approach has considerable potential. **Other approaches.** The free electron laser (FEL) mechanism in which coherent radiation is produced by deflection of a relativistic electron beam in a periodic magnetic field²⁷ is in theory scalable to any wavelength. But in practice, FEL devices have only been used to generate far infrared, infrared and visible emission, with progressively more difficult problems arising for operation at shorter wavelengths because of a $\lambda^{3/2}$ scaling of the amplification coefficient. It has been argued, however, that operation in the vacuum UV and XUV down to 50 nm might be achieved with magnetic wigglers²⁸ and that even X-ray operation at 0.5 nm could be contemplated with an electromagnetic wave as the 'optical wiggler'²⁹. Major and costly technical problems remain to be solved, including providing both an XUV resonator and a suitable storage ring or Linac of exceptionally high current, low electron-beam divergence and small electron-energy spread. The result could be a frequency tunable laser of high average power in a continuous train of pulses similar to a synchrotron radiation source. (Low power coherent radiation can be obtained from any source by frequency and angle selection and in the XUV synchrotron radiation sources equipped with undulators have been used for this purpose.)

Sudden transfer of stored excitation from long-lived to short-lived states can avoid the need for high fluorescent power and schemes based on charge exchange collisions³⁰ and laser excitation of metastable states^{31,32} are of interest. X-ray laser action at a photon energy of $\sim 1 \text{ keV}$ is reported to have been obtained using a thermonuclear explosion as the pumping source³³. No details of this work have been published, though it may be surmised that the mechanism would involve photon pumping.

The device is clearly not a laboratory laser and its potential applications are in the area of the United States' 'Star Wars' programme.

Future applications of XUV lasers

If, as expected, laboratory XUV lasers are developed successfully in the next few years they will offer powerful, MW to GW single pulses at photon energies (or wavelengths) from $\sim 50 \text{ eV}$ (25 nm) to perhaps 500 eV (2.5 nm). These lasers will only be available at major laboratories where there are exceptionally powerful optical frequency lasers to create the plasmas. So the likely applications will be scientific rather than technological, with powerful XUV lasers complementing other sources, notably synchrotron radiation and thermal X-rays from laser-produced plasmas. The XUV lasers will be used to best effect where time resolution, intensity, spectral purity and coherence are important. Speculation on future applications is likely to be inaccurate since unforeseen developments are usually most significant. The most probable initial applications will be extrapolations of current lines of research:

Soft X-ray microscopy in biology. Recent work with single-pulse soft X-ray emission from laser-produced plasmas has demonstrated high-resolution (50 nm) images of living cells using contact radiography on soft X-ray resists³. A laser source offers the possibility of holographic imaging with better resolution and three-dimensional information, with important implications for progress in biology.

Diffraction from crystals. Analysis of molecular structures from single crystal Laue diffraction is fundamental to biophysics², but suffers from the lack of any information on the phase of the diffraction peaks. A coherent laser source could, by holographic methods, give extra information, leading to unambiguous structure determination. The problem is that wavelengths an order of magnitude less than those envisaged here are needed to resolve the molecular structures, so although some low resolution work of this kind could be contemplated, the major prizes would remain inaccessible. Single-pulse diffraction measurements on transient phenomena³ would be possible, for example, thermal and chemical transformations in thin films and surfaces—but only on systems where crystal structures were larger than the wavelength.

Material processing. Research into holographic replication of submicron patterns in lithography for application to microelectronics, integrated optics or diffraction grating device fabrication could achieve still smaller scale using powerful XUV laser sources.

Atomic physics. Sub-nanosecond selective excitation or ionization of atomic or ionic beams with an XUV laser would open up new possibilities in the high-resolution spectroscopic study of atoms, ions and molecules and would be particularly appropriate for XUV lasers in the low-energy region ($h\nu \leq 50 \text{ eV}$) with the high laser power being important for adequate single-pulse yields and the monochromaticity for high spectral resolution.

Laser physics and nonlinear optics. There is considerable interest in improving the characteristics of the new lasers, developing resonators, schemes for shorter and more powerful pulse generation (perhaps by injecting harmonically generated picosecond pulses into plasma amplifiers) and exploring nonlinear optical effects for the first time at XUV frequencies.

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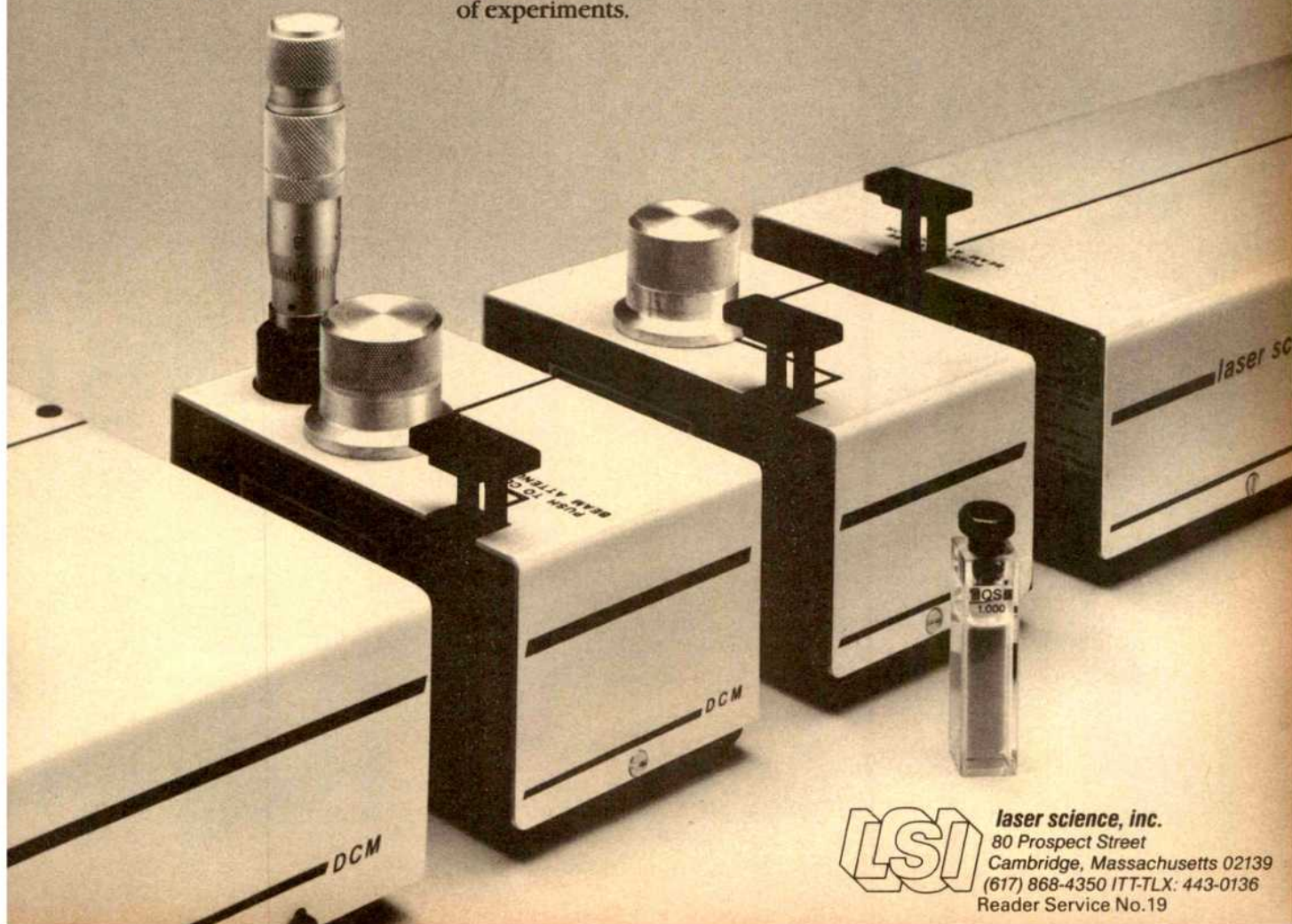
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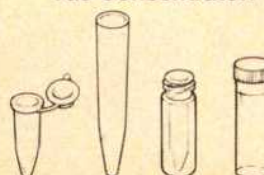
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Lasers, nonlinear optics and optical computers

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Devices whose optical properties change with light intensity have opened the path towards the optical computer. Optical switches and signals can now undertake all binary logic operations and complete optical digital circuits have been constructed.

FROM the earliest days of the laser, the large intensities available in laser beams permitted irradiances of the order of MW cm^{-2} incident on material by simple focusing. With such fluxes the amplitude of the oscillating electric field, E , readily becomes comparable to interatomic fields such that the optical properties of the medium vary with the passage of the wave. The polarization, P , induced by the radiation in the simplest case (of a single monochromatic wave propagating through a crystal of high symmetry) has the form

$$P = \chi^{(1)}E + \chi^{(2)}E^2 + \chi^{(3)}E^3 \dots \quad (1)$$

The susceptibilities $\chi^{(1)}$, $\chi^{(2)}$, $\chi^{(3)}$... are related to the optical properties of the medium: as examples, the linear optical properties are related to $\chi^{(1)}$, the second harmonic generation is determined by the magnitude of $\chi^{(2)}$ and the intensity dependence of the refractive index is related to $\chi^{(3)}$. The science of nonlinear optics developed with the observation of second harmonic generation by Franken *et al.*¹ in 1961 and the theoretical prediction of a series of nonlinear effects by Bloembergen² and co-workers.

The large powers required in the early work made it seem unlikely that digital optical computing, using optical circuitry with nonlinear optical circuit elements, would ever be practical. It was nearly 20 years before the experimental discovery that a 30-mW continuous wave (CW) laser beam could be significantly self-defocused in a narrow gap semiconductor produced the surprising conclusion that third order nonlinearities ($\chi^{(3)}$) could have a value of the order of 10^9 times larger than the nonlinearities envisaged by Franken and Bloembergen^{1,2}.

To understand the significance of this giant nonlinearity to a possible future of optical computing, consider the physics of computation (see ref. 3). Information is essentially stored as energy and switching a device from logic 0 level to logic 1 level requires a definite switching energy. This energy must be greater than the thermal energy of the device kT (and will usually be several hundred times kT) and scales as the size of the device. There will usually also be a trade-off between speed and power. A further absolute requirement for electronic or optical logic is the necessity of restoring a logic level after each switching action so that errors resulting from imperfect devices and signals do not accumulate. Restoring logic must have power gain and this power is normally drawn from a power supply independent of the signal channel. (The requirement of power gain is obviously necessary, that is, one switching device must be capable of

driving at least the next in the series so that a circuit containing multiple elements can be constructed.) These considerations appear to be applicable to all systems of signal processing and computing.

The response of semiconductor micro-electronics to the high data rate requirements of digital signal processing and computing has been to increase switching speeds and further miniaturize components in the form of very large-scale integration (VLSI). Transistors made from gallium arsenide have been reported with effective switching times as fast as 12 ps. However, this will not necessarily solve the problem of coping with high data rate as the processing time in conventional computers is many times the logic switching time because of the necessity of transferring information to the next part of the circuit. This involves capacitance time constant limits as pointed out, for example, in ref. 3; VLSI does not solve the time-constant problem because, as the length of a wire shrinks by a factor α and the cross-sectional area of the wire is reduced by a factor α^2 , the capacitance C of the wire decreases by this factor α while the resistance R increases by the same amount. Thus, the time constant RC remains the same and the input charging time remains unaltered independent of scaling.

Turning to the computer as a whole, the standard method of communication in use today connects the logic unit with the memory through an address device. This reduces the number of interconnections but can only address one storage element at a time. This widely used scheme was first suggested by von Neumann but, rather than being given credit for this most practical innovation, he is now rather undeservedly blamed for this so-called "von Neumann bottleneck". The timing problems associated with circulating logic signals around a one-dimensional processor of this type ('clock skew') combine to indicate that future problems in digital computers are likely to be those of communication. This may apply at all levels (architectural, bus and chip) and stems from the use of time multiplex to compensate for the inability of electrical methods to communicate many channels of information in parallel.

Digital optics

Present practice has seen the invasion of electronically based communication by optical methods through the use of optical fibres in long-range telephone lines. The higher carrier frequency used gives potentially higher bandwidth, although electronic

limitations on modulation techniques have restricted our ability to exploit this greater information carrying capacity fully. Currently, long-range transmission made possible by low signal attenuation has been used. The use of optics for processing information has so far been handicapped by the absence of optical circuit elements. The optical methods have promising implications in areas that are currently difficult for existing technologies; these include image processing and recognition, sorting, radar array signal processing, machine vision and artificial intelligence.

During the past 20 years there has also been a body of work often called optical computing practised by optical researchers using linear processing devices, for example, the use of spatial filters and Fourier transform processes in image processing. Ideas include the use of symbolic substitution, residue arithmetic, vector-matrix multiplication and primitive processing experiments using a liquid crystal light valve as a hybrid (that is, optics with electronics) spatial light modulator but not itself capable of all-optical logic action. Most of the reports are theoretical proposals of what might be done (see ref. 4).

The major stumbling block in the development of digital optics has been the absence of nonlinear all-optical circuit elements, preferably capable of fabrication in the form of two-dimensional arrays and of small enough size to have small switching energies and high speeds.

The main purpose of this article is to review the latest state of research in all-optical nonlinear logic switches, amplifiers and memories and to show that, with the experimental realization of the first all-optical circuits, the time is now ripe for the combination of these two previously separate research efforts, optical bistability and linear optical computing techniques.

All-optical circuit elements

Recent progress, which has produced practical all-optical circuit elements, is based on the combination of optical nonlinearity and feedback. This has led to the concept of 'optical bistability' and hence to a whole family of devices based on a common set of physical and mathematical principles. The series of devices includes optical logic gates, bistable memories, amplifiers (sometimes termed optical transistors or transphasors) and power limiters (see ref. 5 for a recent Royal Society, London, conference). The meeting of the International Commission of Optics in Sapporo⁶ presented six papers on optical computing and began the process of introducing the optical bistability community to the optical information processors. The recent Optical Society of America conference⁷ at Lake Tahoe continued this process; a three-element loop processor, using all-optical interactions, was reported, together with experiments on parallel optical computing, using liquid crystal light valves as the nonlinear elements.

A seminal paper on optically bistable devices was that of Szöke *et al.*⁸ who in 1969 proposed that a Fabry-Perot optical resonator containing a saturable absorber as its spacer layer could exhibit two states of transmission for the same input intensity. This simple bistable action was predicted to arise from the existence of a high internal optical field at constructive interference given that sufficient intensity had been incident on the resonator to bleach the absorber. To reach this condition required a greater input intensity than that required to maintain it. By contrast, at low input intensity the non-bleached absorption held the transmission of the device at a low level. In practice this condition is quite hard to achieve experimentally and the experiments described do not in fact show optical bistability. Observation of optical bistability was not made until 1976 when Gibbs *et al.*⁹, using an interferometer containing sodium vapour, observed bistable transmission but deduced that the dominant mechanism was refractive, involving a shift in resonator frequency, rather than absorptive. Nevertheless, effective refractive nonlinearity resulted from a saturation of the atomic absorption. Such a device, although using only milliwatts of power, was relatively large (centimetres in length) and relatively slow (milliseconds) compared with electronic circuit components.

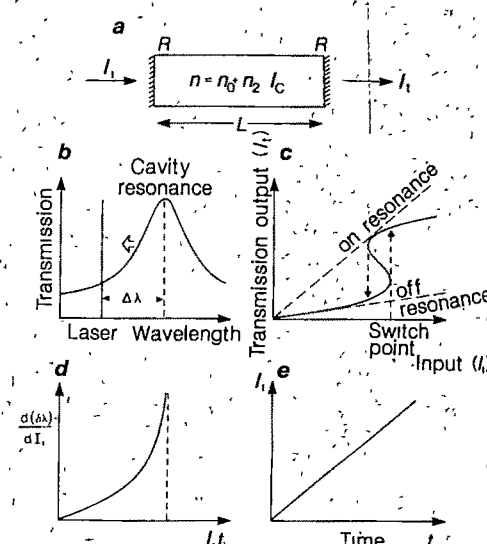


Fig. 1 The dynamics of switching in a Fabry-Perot etalon (see text for explanation of individual diagrams).

In the same year, my colleagues and I made the surprising discovery of giant nonlinear refraction, defined as a function of the intensity, I , from

$$n = n_0 + n_2 I \quad (2)$$

(where the nonlinear refractive index n_2 can be measured in units of cm^2 per kW) present in the narrow bandgap semiconductor InSb. The immediate implication was that a bistable resonator could be made of micrometre dimension and, as the effect was shown to be electronic, would be fast, probably on a nanosecond timescale. A second implication was that one beam could modulate the optical properties of a small slice of semiconductor and affect a second beam, thus making an optical modulator or optical transistor. The details of both the refractive and associated absorptive nonlinearities at milliwatt powers are described in ref. 10 but, retrospectively, the effects could be recognized in the earlier work on the spin-flip Raman laser. Both the device possibilities described above had been practically realized in InSb by 1979 (ref. 11) in which continuous wave laser beams were used, leading to steady-state operation and true optical bistability, as well as the observation of gain in an optical transistor¹².

Simultaneously and independently, optical bistability was reported by Gibbs *et al.*¹³ using pulsed dye laser radiation in the larger gap semiconductor GaAs. Larger absorption and a smaller nonlinearity in this material, however, prevented steady-state operation and this observation was therefore quasi-dynamic and did not permit the demonstration of logic levels or differential amplification.

Origin of giant nonlinearities

The physical explanation of the large nonlinearities in both these semiconductors involves the excitation of electrons to give some degree of saturation or 'blocking'. In the case of InSb, exciton effects are negligible in the conditions of the experiment and a plausible explanation has been given by Miller *et al.*¹⁴ in terms of a 'dynamic Burstein-Moss' shift of the band edge. Physically, a number of electrons ($\sim 10^{15}$ – 10^{16} cm^{-3}) are excited into lower conduction states by the laser photons. The incident intensity I generates an equilibrium number of electrons. Subsequent band filling, following thermalization, modifies the absorption edge and by application of the Kramers-Kronig relationship causes a change in refractive index,

$$\Delta n = \frac{\hbar c}{\pi} \int_0^\infty \frac{\Delta \alpha(\hbar \omega')}{(\hbar \omega')^2 - (\hbar \omega)^2} d(\hbar \omega') \quad (3)$$

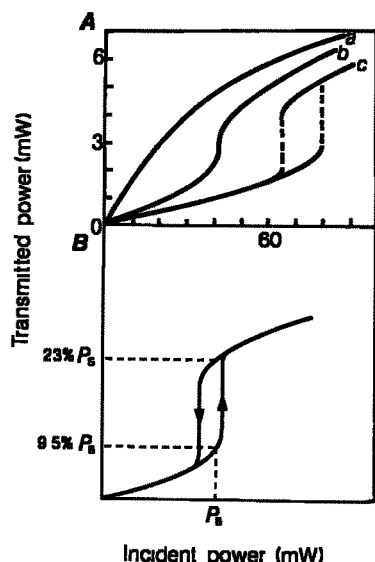


Fig. 2 **A**, Experimental observations of a family of characteristics of an interference filter bistable device illuminated by the argon ion laser line of wavelength 514 nm, obtained by changing the initial detuning from resonance of the etalon. The family of curves *a-c* shows the characteristics obtained for different values of the initial detuning parameter $\Delta\lambda$ set by the angle of incidence. If the initial condition is close to resonance, power limiting action is obtained (*a*) as the transmittance can only fall as intensity rises. As $\Delta\lambda$ is increased, curve *b* begins to kink, showing a greater change in output than for an incremental change in input, and thus exhibiting differential gain. This is responsible for optical transistor or transphaser action. Further increase of $\Delta\lambda$ leads to bistable loops of varying width (*c*). **B**, Experimental observation of optical bistability in the interference filter illuminated by light of wavelength 528 nm. The stability of the system is sufficient to allow operation to within $\sim 4\%$ of the power required to switch

where $\alpha(\hbar\omega')$ is the interband absorption coefficient at photon energy $\hbar\omega'$. With $\Delta n/\Delta I \approx n_2$ we can obtain an analytical expression for n_2 . This calculation gives a good description of the resonant refractive nonlinearity in InSb, InAs and CdHgTe.

Device physics

The simplest configuration which provides optical feedback is a simple Fabry-Perot etalon containing a nonlinear refractive material (Fig. 1a). Its optical thickness is given by

$$nL = (n_0 + n_2 I_c) L \quad (4)$$

(where L is the thickness of the material) and this changes with the internal intensity I_c . Consider now the transmission of such an interferometer as a function of wavelength (Fig. 1b): if we start in an initial condition where the illuminating laser wavelength is detuned from maximum transmission by a wavelength increment $\Delta\lambda$ (Fig. 1b), we see from Fig. 1c that the relation between output and input would give rise to, in the case of a linear device, a straight line of low slope: if the device were tuned to resonance and there were no absorption loss, output would be related to input by a line at 45° . If we now increase the intensity from the initial condition, the nonlinear resonator tends towards resonance as its optical thickness changes with intensity. This gives rise to a nonlinear relation between output and input. However, as we approach resonance, the internal field circulating within the resonator itself builds up according to

$$I_c = I_i T(\lambda) (1 + R) / (1 - R) \quad (5)$$

where I_i is the incident intensity, $T(\lambda)$ is the transmission as a function of wavelength (as in Fig. 1b) and R is the (constant) reflectivity of the resonator mirrors. Thus, at resonance the internal intensity is at its maximum where $T = 1$ and is amplified by the term $(1 + R)/(1 - R)$. This gives rise to positive feedback. As resonance is approached, the internal field builds up and

the rate of approach to resonance depends on the change in optical thickness which itself depends on the magnitude of the internal field. The rate of approach to resonance thus speeds up. This can be readily expressed through the expression

$$\frac{d(\Delta\lambda)}{dI_i} = T(\lambda) / \left(\frac{T_{\max}}{2n_2 L} - I_i \frac{dT}{d\lambda} \right) \quad (6)$$

Figure 1d shows a plot of the rate of approach to resonance as a function of either incident intensity I_i (or as a function of time if we assume a linear ramp of I_i against time; Fig. 1e). As I_i increases, $dT/d\lambda$ also varies, the denominator of equation (6) tends to zero and the rate of approach to resonance becomes infinite, leading to rapid switching. This gives a physical feel for the dynamics of the switching process and leads to optical bistability (Fig. 1c), that is, two values of the transmission for one of the intensity. Two features are noteworthy: (1) a large value of finesse (ratio of spacing to half-width of the transmission peaks) makes it easier to obtain bistability; and (2) the form of the characteristic (output versus input) changes with the initial conditions, that is, it depends on $\Delta\lambda$.

Such a set of characteristics is illustrated in Fig. 2, giving a set of experimental results for nonlinear interference filters incorporating ZnSe films as the active layers (curves *a-c*). Thus, by a simple change of initial conditions a whole family of optical devices can be produced.

Figure of merit for optical devices

An analysis of the factors concerned in the design of such optical circuit elements has been given by Miller¹⁵, who shows that the quantity which gives a figure of merit (in terms of nonlinear Fabry-Perot cavity optical switching in the presence of linear absorption, α) is $n_2/\lambda\alpha$. This determines the lowest critical value of input irradiance I_c for a device of given size to obtain bistable switching or nonlinear characteristic:

$$I_c = \frac{\alpha\lambda}{n_2} f(R, \alpha L) \quad (7)$$

The result is physically sensible as the switching power will be lower for a larger nonlinearity n_2 , the shorter the wavelength the smaller will be the refractive change required to effect a change from constructive to destructive interference (that is, $\Delta(nL) = \lambda/2$) and for a smaller absorption (assumed linear in this analysis) the longer the device may be for a given loss. The function f optimizes cavity properties. Values of $n_2 = 0.1 \text{ cm}^2 \text{ kW}^{-1}$ and $\alpha = 10 \text{ cm}^{-1}$ give useful devices of thickness (L) 50–200 μm , bearing in mind that a fractional change $\Delta n/n \sim 10^{-3}$ is usually required.

To obtain favourable values for $n_2/\lambda\alpha$, we make particular use of effects resonant with the energy gap E_G in semiconducting materials. For electronic nonlinearities it can be shown that $n_2 \sim 1/E_G^3$ so that $I_c \sim 1/\lambda^2$. In addition, the considerations made earlier suggest that the smallest possible devices should be constructed. Diffraction limits suggest that the area limit will be $\sim (\lambda/n)^2$ and thus, although the nonlinearity is clearly large at longer wavelengths for small gap materials, the interference conditions and device size favour shorter wavelengths. Analysis of the detail of the frequency dependence of the nonlinearity, together with the attendant unwanted losses, has not so far indicated an optimum wavelength.

Semiconducting compounds have shown promising results: InSb with a typical working wavelength (5.5 μm) corresponding to $1,820 \text{ cm}^{-1}$ is one material for which there are sufficient available laser frequencies to undertake a detailed examination of the frequency and hence resonant behaviour of n_2 and α near the band gap^{5,14}.

GaAs has also been investigated^{5,13} but differs by having a strong exciton feature near the absorption edge. The nonlinearity $n_2 \sim 10^{-3} \text{ cm}^2 \text{ kW}^{-1}$ is quite practicable for devices, but the absorption coefficient in epitaxially grown material is so far giving values of $\alpha \sim 10^4 \text{ cm}^{-1}$. Thus, thicknesses are restricted to a few micrometres and thermal stability poses a problem.

Both the above materials show negative values of n_2 caused by electronic effects. Carrier lifetimes are in the range of tens to hundreds of nanoseconds. The nonlinearities can be 'switched on' more quickly than this time interval by rapidly introducing carriers with relatively intense pulses. The relative figures of merit between GaAs and InSb favour InSb by a factor of 10^5 , which has given InSb the advantage of steady-state operation but the disadvantage of a low operating temperature of 77 K, whereas GaAs has operated at room temperature and at shorter wavelengths. So far there are no reports of true steady-state operation for GaAs material. Other materials, which have been used in purely optical switching devices, are reported in ref. 5.

There is a second useful form of nonlinearity involving thermal shift of the band edge resulting from temperature rise of the bulk of the nonlinear material. This effect also resonates with the band edge and is associated with moderate values of absorption coefficient. The quantity taking the place of n_2 is an effective $n_2 = (dn/dT)\alpha(LL'/\kappa_s)$ where L, L' introduce dimensions of film and substrate thickness and κ_s is the thermal conductivity of the substrate. Interference thin film structures using ZnSe (ref. 16) have given promising results for refractive switching, and bulk ZnSe, CdS and GaAs also show 'optical bistability by increasing absorption' where the thermal shift introduces the required feedback⁵.

Requirements for optical computing

Since our proposition is to undertake logic operation by means of these optical circuit elements, we can define some of the requirements if they are to be put together in the form of optical circuits to construct an optical computer. Requirements are as follows: (1) High contrast. A logic device needs to show a large change between logic 0 and logic 1 levels. (2) Steady-state bias. To make various different logic gates it is necessary to alter controllably optical bias levels. In terms of optical bistability this means that the device can be 'held' indefinitely at any point on the characteristic with a CW laser beam—the 'holding beam'—and implies a degree of thermal stability. Devices based on InSb at 77 K and on ZnSe at 300 K have been the first to show this behaviour. (3) External address. For logic functions it is clearly necessary that separate external signal beams can be combined with the holding beam to switch the device. The switching energy in fact is derived from the holding beam and this switched beam propagates in transmission or reflection as the output beam to the next devices in an optical circuit. (4) The elements must be cascable. This means that the output of one device must be sufficient to switch at least one succeeding device. The ability to set a CW holding beam near to switch point in fact fulfils this condition because the extra increment is then small compared with the change in output even in the presence of loss. As each device has its own 'power supply' (that is, holding beam), logic levels are restored. (5) Fan-out and fan-in. The probable advantages of parallel processing in optical devices emphasize the ability that one device can drive many succeeding devices, probably using free space propagation for address. The summed effect of several elements can readily be focused on one device to achieve fan-in. (6) High gain. Items (4) and (5) demand that the elements show a value of differential gain ratio of change in output to change in input of >1 . (7) Arrays. The technology shall ideally be such that two-dimensional arrays are easily constructed. (8) Power and speed. Low power per device is a necessity and this should preferably be of the order of milliwatts or less; this is aided by fabricating small-area devices. Speed and power will be interchangeable but speed itself will vary with use: for parallel arrays, microseconds will suffice and for one-dimensional circuits, sub-nanosecond or picosecond switching times are desirable.

Experimental results

Figure 3a shows an early result for an InSb resonator operating in transmission. The critical switching power is 20–25 mW incident on a diameter of $\sim 200 \mu\text{m}$. If the device is set up in

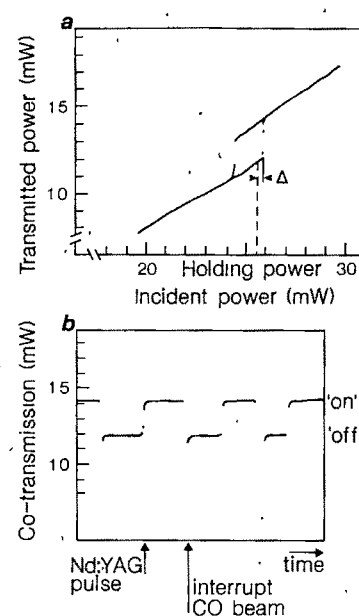


Fig. 3 *a*, Transmission characteristic of an InSb etalon illuminated by a steady-state carbon monoxide laser operating at a wavelength of $5 \mu\text{m}$. The laser power is constant at a level slightly below that required to switch. *b*, The change in transmission induced by the absorption of 35 ps long pulses from a Nd:YAG laser ($1.06 \mu\text{m}$) and total energy 5 nJ.

this way with the holding beam adjusted in intensity to be just short of the switch point, the device may be externally addressed. Figure 3b shows the results obtained using a single 35-ps long pulse of 5 nJ energy from a Nd:YAG laser. The arrival of the single pulse is sufficient to trigger the switching and the device remains in the 'on' state. Interrupting the holding beam returns the switch to the 'off' state, and as can be seen the logic levels are extremely stable. The device was further developed to show explicit AND gate operation by dividing the switching pulse with a beam splitter and observing that the switching energy was quite definite so that the device could be set to switch only when both pulses were incident (in addition to the steady-state holding beam) and would not switch with a single pulse. The device is therefore acting as an AND gate and an optical memory.

We may deduce that sufficient free carriers were induced near the surface of the InSb resonator to cause sufficient change in optical thickness to initiate switching within ~ 3 ps. W. Kaiser (personal communication) has shown recently that this nonlinearity can be switched 'on' in <7 ps. Therefore, the probable switching time is limited only by the macroscopic effect of internal field build-up within the resonator. This depends only on the round-trip time for the $210\text{-}\mu\text{m}$ thick etalon and amounts to ~ 8 ps. We may infer that picosecond timescale optical logic has been demonstrated.

First digital optical circuits

With CW steady-state hold, an InSb logic device can be switched with incremental power as little as $3 \mu\text{W}$ (Fig. 3). Thus, the output change of the device operating in reflection (Fig. 4), ~ 4 mW, should be sufficient to switch a succeeding device. A first experiment using two optical switches, A and B, is illustrated in Fig. 4. This first example of an optical logic circuit is equivalent to an XNOR gate.

Thin-film interference structures comprising alternate layers of high- and low-index material deposited on a flat substrate such as float glass are a convenient way of constructing a resonator equivalent to the devices described earlier. If sufficient nonlinearity can be induced in the very thin interference layers the technology has many advantages, particularly that very uniform devices covering many square centimetres can be easily made. Thus, large-area arrays are readily possible. Typical total thicknesses of films for a device with 15 layers are only $\sim 2\text{--}3 \mu\text{m}$.

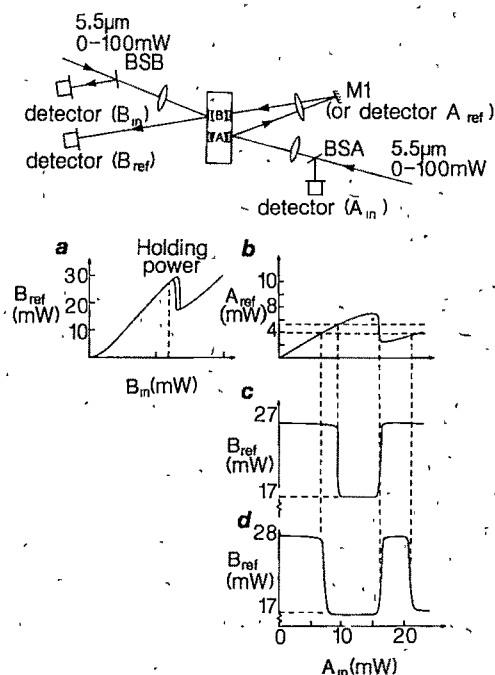


Fig. 4 The experimental arrangement used in the fabrication of an XNOR gate by the coupling of two InSb logic gates and the characteristics of the individual (*a*, *b*) and coupled (*c*, *d*) gates. Optical switch A is addressed by a holding beam which is reflected from the element and focused on to a second element, B, 500 μm away on the same crystal slice. This second element is held near to switch point by a second holding beam illuminating it from the opposite side. The output of A then acts as the address beam for B and the optical circuit output is observed as the reflection from B (B_{ref}). The figure also depicts the circuit inputs and outputs as the input to A (A_{in}) is steadily increased until a point is reached where its reflected output acting as address on B causes this second gate to switch to a lower state in reflection (*c*), (*d*). B remains in this lower state until A itself switches down in reflection, when B simultaneously switches up in reflection. B remains in the up state as the input to A is further increased until it again switches down when the reflected input from A is high enough. If A were to be held close to switch point and addressed with a pulse, information would be stored in A. If the holding beam on B is at the same time too far from switch point, the information will not be transferred from B. If this holding beam is then programmed to come within the range where B can be switched, the information can be transferred to B and by an appropriate reduction of the holding beam on B the information can be moved from A. The device can therefore be made to act as a shift register. With further holding beams defining optical circuit elements across the crystal, these forms of optical logic become indefinitely extensible.

The heat capacity of pixels of micrometre dimension is low and implies that incident power of ~ 10 mW can cause a temperature rise of 50°C in ~ 1 ns. The important physics lies in the control of heat sinking by means of the conductivity of a relatively massive substrate. Figure 2 shows the very promising results obtained using ZnSe as the active film which has a band gap conveniently resonant with argon ion laser wavelengths at 514 and 528 nm. The important property recently found is that these logic switches are sufficiently stable to permit construction of optical circuits of a similar nature to those already demonstrated with InSb^{5,17}.

Optical computers

I have described the contemporary performance of several optical circuit elements and shown that, in principle, all logic functions familiar in electronics can be reproduced by these nonlinear optical devices. Therefore, it should be possible to construct an all-optical computer.

There exists a considerable body of literature reporting the research of a number of groups on the subject of optical comput-

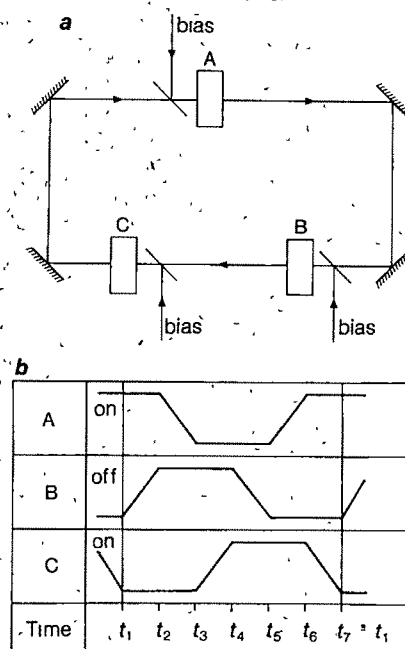


Fig. 5 *a*, A schematic model of the arrangement used to demonstrate a three-gate optical delay which forms the basis of a loop processor. *b*, The clocking of the individual bias levels to the three gates.

ing⁴. To date this has not included the use of the nonlinear elements discussed here. Nevertheless, the advantages of using optical methods for various computations have been apparent for several years. Historically, most optical computing systems have been of analog type in which information is stored and processed as a continuum of signal levels. There are major drawbacks to such analog systems, including limited flexibility, noise accumulation and input/output device limitations. The advantages of optical binary logic, now in principle possible with the devices described here, may be crucial in progressing optical computing into a new practical technology. One would wish to avoid as many photon-electron or electron-photon conversions as possible. The considerations can be made at several levels, including individual optical logic devices (gates and arrays of devices) as the first level. The second level considers optical communications interconnections and input/output among the logic gates, among arrays and among circuit boards or processors. The third level considers the possibilities of new computer architecture to take advantage of the inherent parallelism of optics.

Thus, one might see the advantages of optical methods in two separate ways: (1) Speed of switching and communication. I have shown that individual optical logic operations can almost certainly be performed on a picosecond timescale. Combining this with the output characteristics of CW mode-locked lasers, it is possible to conceive bit rates approaching frequencies of THz for an exact analogue of electronic digital logic. It is not yet clear that recovery times can be imposed on the optical logic switches at acceptable power levels to ensure continuous operation. The possibility exists of series-to-parallel and parallel-to-series conversion on picosecond timescales. Fast, high bandwidth communication will also be consistently available. Optical methods may defeat clock skew. (2) Parallelism and communication. The logic devices described here are at this time the only ones which have been capable of providing steady-state information holding and cascable operation. They have time constants in the range 100 ns–100 μs . This suggests that the first approach to the use of optics should emphasize the use of parallelism and accept cycle rates similar to existing electronic speeds. The intriguing intellectual challenge is to use the flexibility of optics to provide appropriate interconnections:

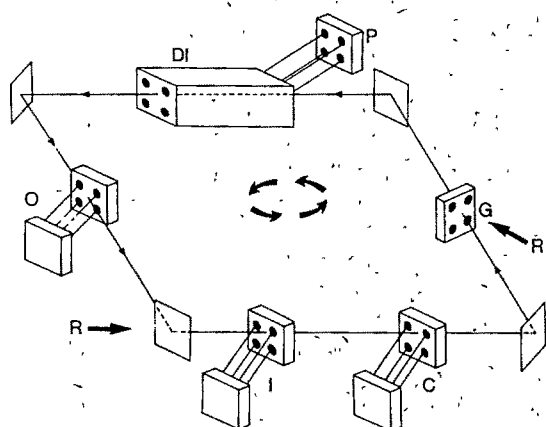


Fig. 6 The types of optical computer being proposed use the parallelism of optics by using arrays of elements. The iteration present in the type of problem which could be solved on such a computer is achieved through the use of a loop processor architecture. The individual arrays of elements may have to be flexible in their uses in the processor, identical arrays may be used as input (I), clock (C), gain (G), program (P), dynamic interconnect (DI) and output (O). Each array may require a separate power supply refresh (R).

requirements could be to shift a logic array pattern by one element on each cycle or to achieve a 'perfect shuffle'. Other schemes include a vector-matrix multiplication involving a fan-in from a matrix array to a column followed by rotation of this column between output and input for successive cycles.

In my laboratory a number of optical-computer architectural components have been designed including memory units, clocks, a programmable processing stage and a simple full adder using simultaneously the transmission and reflection from an optical gate¹⁸. The necessity to store the calculation before communicating to the next element is important in the optical case: propagation at the speed of light otherwise would mean that all elements would be simultaneously addressed (admittedly, avoiding clock skew³). Delays can be readily implemented by suitably programming the holding beams so that the optical bias is only set to receive a signal when the device is clear of other signals. As a first step, consistent with the elements described, this could be achieved electronically making an interesting interface to existing computer technology. At the time of writing, a three-element loop processor with optical bias delay loop clocking has been implemented. Acousto-optic modulators, controlled by a micro-computer, provide both optical bias levels and input data pulses (Fig. 5). In principle, indefinitely extensible optical logic has been demonstrated. A generalized optical computer using these ideas may take the form shown in Fig. 6.

Conclusion

One may conclude that practical optical circuit elements have been demonstrated, that all binary logical operations can be performed and that the first digital optical circuits have been shown to be practical.

At this time the most favourable method of applying optical methods to digital optical computing seems to be the use of digital arrays of gates speaking (in an optical sense) to further arrays of gates with the possibility of fixed or variable-intercom-

munications, fan-out and fan-in using free space propagation as efficient ways of 'optical wiring'. It remains to be shown that holding powers and switching energies are sufficiently small to allow operation with existing lasers and devices. Scaling from present experimental results, one may deduce that, for a visible wavelength device, a single logic element area of $\sim 1 \mu\text{m}^2$ should be practical, which, with a 250-ns switching time, should require 0.2 mW of holding power. Perhaps 10% of this would be absorbed. The 10 W available in the 514-nm argon ion line from a typical commercial laser would allow the operation of 5×10^4 gates assuming perfect optics. This would give the array a rate of information processing equivalent to 2×10^{11} logic operations per second assuming that all the gates are used simultaneously. A similar number of logic operations could be achieved by using small-gap semiconductors in the infrared where the electronic processes would allow faster switching but the packing density and device size would be greater. The optimization of the existing devices and the use of different materials may allow the synthesis of arrays capable of much higher data rates.

Even allowing for imperfections, which would reduce these numbers, the data rates are sufficiently high to encourage the exploration of their use for tasks where conventional one-dimensional sequential digital electronics finds difficulties, such as pattern recognition, artificial intelligence, sorting and specialized computational problems. Equally interesting will be applications such as power limiters, optical noise reduction and laser projectors and displays.

International development programmes

Several collaborating groups are actively involved in research in these areas. The principal effort in Europe is the European Joint Optical Bistability Program, a multinational project on optical logic circuitry and the basic physics of optical bistability involving eight universities and institutes in Britain, Belgium, West Germany, Italy and France. There are a further eight European laboratories associated with the programme. The research support scheme was established in 1984 by the Commission of the European Communities, through its Committee for the European Development of Science and Technology.

In the United States there are two main collaborative groups: The Optical Circuitry Cooperative is currently being established by the University of Arizona, which is involved with over 10 American companies with an interest in optical circuitry. The five-year programme is funded by these companies, the university and a NSF grant. The Pentagon recently announced the formation of a consortium of nine research organizations, including seven universities which will be funded as part of the Strategic Defense Initiative to produce an optical computer.

Japan's Optical Computer Group was formed in 1983 with the purpose of providing the opportunity for its 75 members to exchange information and ideas in the field of optical computing. The group has four to six meetings every year and publishes a newsletter (*Opcom news*) between meetings. The group is associated with the Optics Division of the Japanese Society of Applied Physics with members from Japanese universities and companies.

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Laser biology and medicine

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The substantial repertoire of laser radiation—its coherence, range of intensity and frequency, controllability of focal area and of beam length—has been comprehensively explored in the contexts of micro- and macro-diagnostics of cells, biochemical kinetics, therapy and surgery.

TWENTY-FIVE years have passed since the first laser, whose underlying principles were described by Basov, Prokhorov, Townes and Schawlow^{1,2}, was created³. The main applications of lasers were immediately forecast, but the widespread subsequent developments, such as diagnostic, therapeutic and surgical tools in biology and medicine, were not foreseen.

Biomedical laser applications have already been examined in a series of reviews⁴⁻⁸. The present review, timed to coincide with the 25th anniversary of the laser, surveys some recent developments in this field and outlines the new possibilities of laser techniques in disciplines such as photobiology and photo-medicine, concentrating on the main trends in development.

All biomedical laser applications are based on the interaction of laser light with biological substances. Such interaction causes a broad spectrum of effects, so lasers can be used in a wide range of fundamentally different applications (Fig. 1). In the simplest case, low-intensity laser light is absorbed, reflected or reradiated (as fluorescence) by the substance so that no changes occur within it. Such interactions form the basis for diagnostic laser applications in biology and medicine, both for diagnosis at the atomic or molecular level (spectral diagnostics) and at the macroscopic level (such as motion and shape of cells and organs).

Ultraviolet and visible radiation can excite electronic states in many biologically important molecules leading to photochemical transformations at the molecular level. Such processes occurring under the effect of light from incoherent sources are studied by photobiologists and find practical application in photomedicine^{9,10}. The use of laser light in these disciplines gives several benefits, first, it enhances the speed at which molecules are excited, making it possible to probe photobiological reactions on a picosecond timescale¹¹ and second, it excites higher electronic states in the target molecules through multiphoton processes.

High-intensity laser radiation can cause damage to matter through various mechanisms, for example, liquefaction, intensive heating, evaporation and dielectric breakdown. Such processes, rarely observed under the effect of incoherent light sources given their relatively low intensity, now underlie laser material processing. The same physical processes have proved effective in laser-beam surgery.

Fundamental to these diverse applications of laser light is its unique properties: (1) spatial coherence, allowing laser radiation to be focused and introduced into an optical fibre; (2) monochromaticity or temporal coherence, allowing laser spectral analysis and the resonance excitation of certain molecules; (3) high intensity (or temperature), providing a high concentration of light energy in the substance being irradiated, thus giving

rise to multiphoton processes, heating in the substance and other processes; (4) 'compressibility' into ultra-short pulses, allowing the study of very fast (subpicosecond) primary photoprocesses and the excitation of high-energy levels in molecules at a rate exceeding their relaxation rate¹²; (5) tunability in the range from the infrared to the ultraviolet, which permits selective excitation of certain molecular species. Of course a single laser can provide a combination of several of the above properties, although only one or two are essential to each particular application. I consider here the three areas of biomedical laser applications.

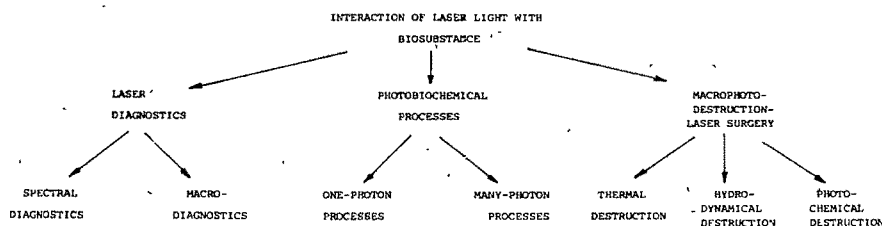
Laser diagnostics

Of importance in biology and medicine are microdiagnostics at the atomic and molecular level and macrodiagnostics at the level of cells and organs. Laser techniques are of great value in both cases.

Atomic and molecular diagnostics. Spectral analyses, such as trace-element analysis in studies of metabolism and toxicology, absorption and fluorescence techniques for the analysis of biological molecules, were performed extensively before the laser was devised. However, spectral analysis without the laser enables the detection of at best $\sim 10^{10}$ atoms or molecules of a particular species in a typical sample. Using lasers, ultra-sensitive analysis techniques^{13,14} were developed which make it possible to detect even single atom or molecule¹⁵. Furthermore the high spectral resolution and selectivity of laser spectroscopy techniques frequently allow the atomic content of samples to be determined directly, without resorting to preliminary separation into components and subsequent chemical analysis. (One example is direct resonance ionization detection of traces of aluminium in human blood¹⁶.) This highly sensitive and selective technique is universal in scope and provides a means of classifying the content of elements in various human organs (down to specific cells) and of revealing their role in human metabolism under both normal and pathological conditions.

This task is inseparable from the analysis of the environment (for example, flora and fauna, foodstuffs) for toxic and pathogenic materials, and the determination of pathways by which they enter the human organism. Here, laser-spectroscopy techniques are especially effective^{13,14}. Even the comparatively simple fluorescence analysis method is highly sensitive when used with the laser, in combination with chromatography. For example, the detection sensitivity for aflatoxins¹⁷ was about 10^{-10} g (10^{-12} mol) until the laser technique was developed; laser resonance photoionization of molecules and mass spectrometry increase the detection sensitivity by several orders of magnitude. Thus, for example, a detection sensitivity as high as 10^{-14} g has been reached for tryptophan in water¹⁸.

Fig. 1 Main trends of laser applications in biology and medicine, based on various degrees of laser light interaction with molecules, from diagnostics to destruction.



The absorption and fluorescence spectra of human tissues under normal conditions differ from those under pathological conditions, a phenomenon long used in diagnostics. The use of laser light, which can be easily delivered into the human body and focused on the desired spot, has extended the capabilities of such techniques. For instance, laser-fluorescence bronchoscopy is effective in early lung-cancer localization¹⁹. The technique uses a sensitizer, haematoporphyrin derivative (HpD), which concentrates predominantly in tumour cells. This compound absorbs light in the 400-nm region and fluoresces in the red region, allowing the detection of cancer at an early stage. Recently it has been shown that the fluorescence spectra of normal and atherosclerotic blood-vessel walls differ appreciably²⁰, a phenomenon which may prove valuable in operative diagnosis in blood-vessel surgery, an area of particular importance in view of the prospects for the development of laser blood-vessel surgery (laser angioplasty), discussed below.

Optoacoustic spectroscopy may, in future, be used to localize intraocular malignant melanomas²¹. The technique involves the generation of ultrasound at a spot inside the eye by focused laser radiation and detecting inhomogeneities by monitoring both the generation and propagation of the sound.

The development of fibre optics and the subsequent creation of fibre-optic sensors for biomedical applications²² have extended the capabilities of the laser spectral diagnosis of internal diseases. Such sensors have already been successfully used for fluorometric analysis, but possibilities here extend much further, because optical fibres allows transmission of light to the desired spot inside the human body not only in therapeutic but also in higher, surgical, radiation doses.

Laser radiation can be focused into a small spot of the order of the radiation wavelength, λ , in size. This property is being exploited in a range of techniques for the microspectral analysis of biological objects. For example, focusing an intense laser pulse onto the surface of a biological object can help to evaporate a $\sim 1\text{-}\mu\text{m}^3$ microsample and produce atomic and molecular ion species in the process; mass analysis of these species can provide information on the atomic and molecular composition of the sample²³. This principle underlies the commercial laser micromass analyser (LAMMA) manufactured by Leybold Heraeus.

Using a tunable laser radiation of a lower intensity allows the study of Raman scattering, absorption and fluorescence spectra of small areas of material without its being destroyed. This forms the basis, for example, of the laser microfluorometry of the processes occurring in single cells or organelles in the living state, the spatial resolution is as high as $0.3\text{ }\mu\text{m}$ and temporal resolution, 0.2 ns (ref. 24). Such a technique could be useful in the fluorescence mapping of genes. One possible technique for the direct observation of the primary structure of DNA is by means of a combination of the selective laser ionization of molecular chromophores with ion-field microscopy²⁵. This technique, which I and my colleagues have been working on for 10 years, would enable analysis of genetic information of individuals and early diagnosis of hereditary diseases.

Currently at the development stage are laser techniques for detecting rare radioactive isotopes, based on the isotope-selective detection of single atoms²⁶ and independent of the radioactive decay process itself. Such methods can be used as the basis for isotope analysis *in vivo* of metabolic pathways in an organism using rare isotope tracers of ultra-low radioactivity. In this context, it would be possible to conduct experiments, even at the cellular level, at a harmless radioactive dose.

Lasers can produce powerful ultra-short light pulses with a duration in the range 10^{-9} – 10^{13} s and, recently, in the femto-second range (down to $20\text{ fs} = 2 \times 10^{-14}\text{ s}$) (ref. 27). Picosecond light pulses have found wide applications in the investigation of primary processes involved in photosynthesis, vision and biochemical reactions involving haemoglobin, DNA and other biologically important molecules, which occur after absorption of a photon^{11,28}. This application of lasers has developed into a vast area of research, providing biochemists with direct and

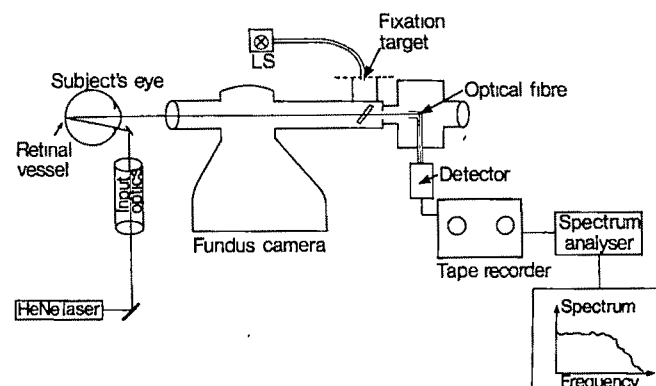


Fig. 2 Scheme of optical and electronic arrangement used to measure Doppler-shifted spectra from human retinal vessels. The basic optical component is a standard fundus camera modified with laser-input optics, internal fixation target and an optical fibre mounted in a scanning ocular (from ref. 32).

reliable information on ultra-fast primary photoprocesses for which only indirect data have been previously available.

Macrodiagnostics. The high monochromaticity and coherence of laser radiation allow measurement of the position, velocity, small displacements and shapes of various components of biological objects, that is, to perform macrodiagnostics for biomedical purposes.

One of the first effective biomedical laser applications was in flow cytometry, where the laser was used to effect the rapid analysis and separation of individual mammalian cells^{29,30} by accurately measuring their optical properties (particularly laser-induced fluorescence characteristics). The cells in suspension exist in a fast-moving, very thin laminar stream which is irradiated by a laser. When a cell of the desired species crosses the laser beam, it begins to fluoresce. The fluorescence triggers a system that diverts the droplet containing the cell. Such laser fluorocytometers are now available commercially; the next step will be to develop a technique for separating male and female cells by the slight differences in their fluorescence spectra resulting from differences between their sets of chromosomes.

Another effective laser application is the measurement of slow fluid flow velocities, particularly blood-flow velocity in vessels. The technique is based on the Doppler shift of the output frequency of a monochromatic laser (usually a He-Ne laser operating at $\lambda = 6,328\text{ Å}$), which occurs as the laser radiation is scattered back from micron-sized particles moving in the blood flow. For example, at a flow of $v = 1\text{ cm s}^{-1}$ the frequency shift, $\Delta\nu$ of the He-Ne laser is $\Delta\nu = V/\lambda = 1.58\text{ kHz}$ and can be detected by monitoring the beat signal on a photodetector receiving both the incident and reflected (back-scattered) radiation. Laser Doppler velocimeters allow for continuous non-invasive blood-flow velocity measurements at the capillary level³¹. As an example, Fig. 2 illustrates schematically the direct measurement of retinal blood-flow velocity in the eye³². The eye is especially convenient for such measurements as its natural optics make it easy to deliver light to the desired microcapillary with an irradiation region diameter of only $100\text{ }\mu\text{m}$. However, the development of fibre optics has made this technique applicable to other blood vessels³³. These fibres enable the measurement of blood-flow velocity profiles with a resolution of 0.1 mm or better, and velocity variations lasting $\sim 0.01\text{ s}$ or less²². By such means it will be possible to measure microcirculatory blood flows with high resolution using an optical fibre brought into contact with the tissue of interest. The development of compact diode lasers should eventually make this technique available for general medical applications. Moreover, the capabilities of the laser-Doppler technique extend much further, for example, it can be used to measure the mobility of bacteria and spermatozoa and to perform electrophoresis of cells and organelles³⁴.

With the advent of lasers, one of the most important pre-laser discoveries in optics, holography³⁵, has become a powerful

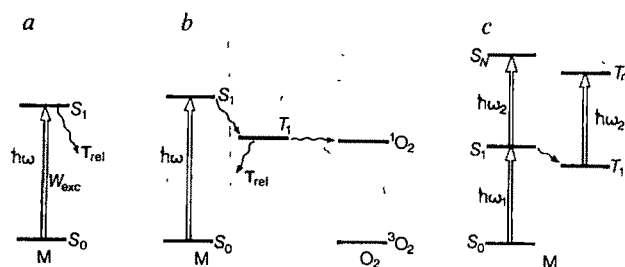


Fig. 3 Methods of photochemical modifications of biomolecules. *a*, One-photon excitation and subsequent photochemical reaction (phototherapy of neonatal jaundice by means of photodecomposition of bilirubin). *b*, One-photon excitation of sensitizer molecule, *M*, and transfer of excitation to modifiable molecule or molecules participating in modification photodynamical effect (tumour phototherapy; the oxygen-acceptor of excitation participates in oxidation). *c*, Two-photon excitation of highly lying triplet, T_1 , or singlet, S_1 , states and following photomodification or transfer of excitation to other molecules (nucleic acids in water).

diagnostic method. Using holography it is possible to obtain three-dimensional images of biological objects; their contours can be mapped (holographic topography) and their deformations can be analysed on a real timescale (holographic interferometry). These new possibilities have been influential in many areas of medicine, including orthopaedics, radiology, ophthalmology, urology and otology³⁶.

Femtosecond light pulses are compressed in space (a pulse duration of $50 \text{ fs} = 5 \times 10^{-14} \text{ s}$ corresponds to a pulse length of $15 \mu\text{m}$ in air). Thus, the possibility arises of direct optical measurement of small distances on the principle of a pulsed optical radar. This technique is applicable to investigations with micrometre-scale spatial resolution into biological systems such as the interior of the eye or skin³⁷. When combined with fibre optics, the technique may have interesting applications in studying human internal organs.

Molecular photobiology and photomedicine

Within this vast area lie those laser applications which make use of photochemical processes resulting from photo-excitation of biological molecules (Fig. 1). To outline what laser light can provide in this area, I consider general schemes of molecular photoexcitation following adsorption of one or more photons (Fig. 3). When being acted on by low-intensity light, a molecule can absorb no more than one photon because the excitation rate $W_{\text{exc}} = \sigma I$ is much lower than the relaxation rate $1/\tau_{\text{rel}}$ of the excited molecule (σ is the excitation cross-section, I the radiation intensity in photons/cm² s, and τ_{rel} the excitation-relaxation time). The excited molecule either takes part in a chemical reaction (Fig. 3*a*) or transfers its excitation to another molecule participating in a chemical transformation (Fig. 3*b*). However, under the effect of short laser pulses of intensity I is so high that $W_{\text{exc}} \approx 1/\tau_{\text{rel}}$, the molecule can absorb a second photon either from a triplet state or from an excited singlet state (Fig. 3*c*). The excited molecule again can suffer both a photochemical transformation and transfer excitation to another photochemically active molecule. The first two schemes form the basis for linear, one-photon photobiology⁹, while the third (and, perhaps, schemes involving the participation of more than two photons) underlies nonlinear, multiphoton photobiology^{12,38}. These two approaches impose different requirements on the laser intensity.

One-photon excitation. One-photon photobiochemical processes, such as phototherapy of neonatal jaundice (hyperbilirubinaemia)³⁹ (Fig. 3*a*) phototherapy and photochemotherapy of various skin diseases (Fig. 3*a, b*)⁴⁰ and photochemotherapy of cancer with the aid of the haematoporphyrin derivative HpD⁴¹ are well known in photobiology⁹.

The last case is a classical example of phototherapy following the scheme of Fig. 3*b*. The HpD molecule tends to accumulate

predominantly in malignant tissues. At the same time, HpD is readily activated with visible light and, once excited, transfers its excitation through a triplet state to the oxygen molecules present in the tissues. The oxygen molecules excited to a singlet state ($^1\text{O}_2$) are chemically active and cytotoxic. The entire mechanism is termed the photodynamic effect⁴².

The light intensities required for all photoprocesses are not very high ($< 1 \text{ W cm}^{-2}$), and so they can be performed under the effect of light from incoherent sources. Nevertheless, the use of laser light here is extremely useful for the following reasons. First, laser radiation is easily delivered to the internal organs through optical fibres without significant loss of intensity. This considerably extends the capabilities of phototherapy, allowing one, in principle, to change from the treatment of external organs to that of any internal organ⁴.

Second, the high intensity of laser radiation ensures a high rate of photochemical processes, irrespective of the excitation of high-lying electronic states. This laser light provides a therapeutic dose over a briefer time span than is possible with radiation from conventional sources. It is for this reason that lasers have given a fresh impetus to the development and improvement of established cancer photochemotherapy techniques using ultraviolet and visible radiation⁴¹.

Third, the necessary irradiation dose can be provided by a single short pulse, which is of particular use in those cases where the effect of light on subsequent secondary chemical reactions must be excluded, for example, experiments on ultraviolet photoreactions of psoralen with DNA, which proceed according to the scheme of Fig. 3*b*⁴³.

Psoralen can intercalate between the pairs of bases of double-stranded nucleic acids, forming covalent adducts with the pyrimidine bases on the absorption of a long-wave ultraviolet quantum. If there is another pyrimidine base on the opposite side, it can also form a second covalent adduct on absorption of a second photon by psoralen. The diadduct thus formed acts as an interstrand crosslink. Where irradiation lasts long enough there is always a large proportion of lethal cross-links which make it difficult to conduct experiments *in vivo*. Irradiation with a single short ultraviolet pulse, however, can be made to yield only monoadducts, as the psoralen molecule can absorb a second photon only after relaxing to the ground state through a triplet state; this takes longer than the pulse duration.

It is precisely by this method that the proportion of mono- and diadducts in the psoralen-DNA photochemical reaction have been controlled following irradiation with a short (15 ns) pulse of an N_2 -laser ($\lambda = 337 \text{ nm}$)⁴⁴. When using a pulsed radiation of a shorter wavelength (a KrF laser, $\lambda = 248 \text{ nm}$), which could be absorbed by the nucleic acid bases themselves, the formation of crosslinks occurred between RNA polymerase and DNA⁴⁵. Again, the use of short ultraviolet laser pulses is advantageous. Photodamage can be caused rapidly, but no structural changes are possible during the time of a single pulse.

Recently, a low-intensity (non-coagulative) He-Ne laser radiation ($\lambda = 632.8 \text{ nm}$) has been successfully used in clinical practice for treating wounds and trophic ulcers^{46,47}. The therapeutic effect of red laser light is frequently explained by its coherence. This argument seems unconvincing, however, as the molecular excitation rate W_{exc} is at least a factor of 10^{10} lower than the rate at which the excited molecules lose their coherence in a condensed medium at normal temperature⁴⁸. Experiments are under way to study this biostimulative effect at the level of mammalian cells, yeasts and bacteria, which have shown that the effect is present regardless of the coherence of the light used, provided its wavelength lies in the appropriate range, 600–850 nm⁴⁹. Thus, this biostimulative effect has no specific relation to the laser and should be considered simply a photobiological effect with useful applications to phototherapy.

Multiple-quantum excitation. The idea that multiple-quantum excitation of biological molecules can be produced by ultra-short laser pulses of low energy but high-peak power was formulated⁵⁰ immediately after such processes had been observed in the gaseous phase (see refs 12, 38). An intrinsic difference in the

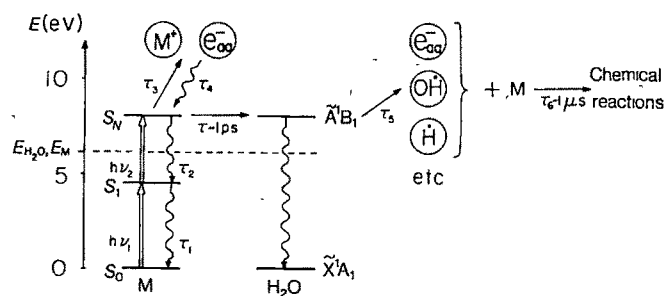


Fig. 4 Simplified diagram of primary photoprocesses occurring in picosecond two-quantum excitation of biological molecules (thymine, a DNA base) in water. Absorption of two ultraviolet photons results in excitation of the S_N Rydberg state lying above the ionization threshold of both the dissolved molecule and water. $\tau_1 = 10^{-12}$ and $\tau_2 = 10^{-13}$ s are the non-radiative relaxation times for the states S_1 and S_N ; $\tau_3 = 10^{-11}$ and $\tau_4 = 5 \times 10^{-12}$ s are the times of formation and recombination of an electron-ion pair; $\tau = 10^{-12}$ s is the time of electronic energy transfer from the dissolved molecule to water. Subsequent ionization and dissociation of water ($\tau_5 = 10^{-13}$ – 10^{-12} s) leads to the formation of reactive radicals. Chemical reactions involving water radicals ($\tau_6 \approx 10^{-6}$ s) are the main photomodification channel for the dissolved molecules.

case of biological molecules in solution is the rapid non-radiative decay of their excited states. The situation is less critical if the molecule relaxes through a triplet state with a comparatively long lifetime (10^{-6} – 10^{-9} s). In this case, a two-step excitation can be effected by means of nanosecond laser pulses (Fig. 3c). However, for the molecule to absorb a second photon from a singlet state, its excitation rate must be close to the relaxation rate that lies in the picosecond range $1/\tau_{rel} \approx 10^{11}$ – 10^{12} s $^{-1}$. Such a two-step excitation can, therefore, be effected only by means of ultra-short laser pulses.

The first successful experiments on the two-quantum excitation of such molecules with picosecond laser pulses and the ensuing photoreactions were conducted with aqueous solutions of nucleic acid bases^{51,52}. As a result of such irradiation, the molecule absorbs two ultraviolet photons ($\lambda = 266$ nm) with a total energy of $2h\nu = 9$ eV which exceeds the ionization and dissociation limit of the molecules of the solvent, water. (Note that the direct one-quantum excitation to the same state ($\lambda = 133$ nm) is impossible because of the strong absorption of water in the ultraviolet.) The highly excited molecules may themselves undergo ionization with a yield that largely depends on the competition between the two-quantum photoionization and recombination processes (Fig. 4). Another possibility is the transfer of excitation to the molecules of the solvent, H_2O , which are subsequently ionized and dissociate⁵³. As a result of this process, the water molecules are decomposed into radicals that react with the dissolved biological molecules. Therefore, when using the two-quantum method to excite high-lying electronic states of biomolecules (for example, thymine) one can observe photobiochemical reactions that usually occur in solution in response to ionizing radiation⁵⁴. However, the ultraviolet picosecond photolysis of biomolecules has several advantages over their γ -radiolysis.

One principal distinction between two-quantum photolysis and γ -radiolysis is that the solvent molecules in the former case are dissociated only near the chromophore molecules which absorb laser radiation, and not within the entire irradiated volume, as is the case with γ -radiolysis. This fact is used to advantage in the laser therapy of tumours, for it provides the possibility of locally producing water radicals that kill tumour cells but have no effect on nearby normal cells, by means of specific sensitizer molecules.

Two-quantum photobiochemical reactions have been observed at various levels of organization in biological systems (for example, in viruses, yeasts and cells)^{55,56} (see ref. 57 for a review). This approach will apply in genetic engineering, especially in combination with microirradiation of cells⁵⁶. For

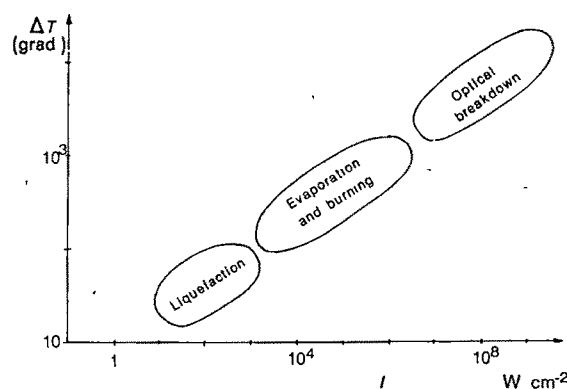


Fig. 5 Various degrees of heating of a substance with the laser radiation that underlie the various laser surgery techniques in ophthalmology.

instance, various types of molecular damage (for example, breaks, crosslinks) have been observed in nucleic acids *in vivo*^{55,57}. This allows the investigation of the spatial organization and functions of complex molecules such as proteins and nucleic acids, especially where effective methods for identifying damage sites are available⁵⁸.

In addition to the direct two- (multiple-) quantum process in biological molecules (following the scheme in Fig. 3c), one can use laser action with the help of sensitizer molecules, which absorb laser photons more effectively. These sensitizers are generally dyes which can form complexes with biological molecules. Once the sensitizer molecule has absorbed enough energy, the biological molecule suffers damage at the spot where the complex has been formed. This method has been investigated in experiments with nucleic acid molecules^{59,60}. For example, polyadenylic acid molecules undergo scission at the point of complexing with the dansyl derivative of oligothymidilate (DDO) under the effect of a powerful N_2 -laser pulse. This phenomenon is caused by the two-quantum excitation of DDO and subsequent transfer of excitation to the nucleic acid⁶⁰. It has been suggested that molecular sensitizers might be used to disrupt nucleic acid molecules at definite, pre-selected points^{60,61}.

The two-quantum excitation of high-lying electronic states is expected to increase considerably the yield of photoreactions compared with the known one-quantum reactions proceeding through triplet states, because the singlet-triplet conversion yield is in many cases much less than 100%. Such an increase in the yield of a photochemical reaction has been observed in experiments on the excitation of porphyrin molecules in solution⁶². Changing from a low-intensity excitation (<1 W cm $^{-2}$) to a high-intensity excitation (10^9 W cm $^{-2}$) increased the quantum yield of photomodification of porphyrin by a factor of 100. Similar experiments with a haematoporphyrin derivative, conducted with the N_2 -laser, showed⁶³ that the primary mechanism responsible for the destruction of this molecule was its two-step excitation. Such excitation produces a stronger cytotoxic effect than low-intensity irradiation.

Summarizing the first results of multi-quantum excitation of biological molecules, we are close to the development of new effective nonlinear laser phototherapy techniques using ultra-short laser pulses capable of producing considerable photochemical effects at an average power too low for thermal effects. In particular, a wide-ranging phototherapy method will probably be developed, based on the transfer of excitation to water molecules⁵⁴, which will extend the capabilities of the existing radiotherapy techniques.

Laser surgery

The use of lasers in surgery is based mainly on the absorption of a substantial amount of light energy in the area being operated on, which causes the heating of the irradiated region and its subsequent destruction by one of several possible mechanisms,

depending on the irradiation conditions. Such photodestruction of the tissue requires no contact, allowing absolute sterility for laser surgery. Second, by properly selecting the laser wavelength, one can attain a certain selectivity of photodestruction, provided that the tissue to be removed differs in absorption spectrum from the surrounding tissues. Third, the thermal effects can be varied over a very wide spectrum, from liquefaction without evaporation⁶⁴ under slight heating to destruction by hydrodynamic shock wave resulting from local intense pulsed heating⁶⁵. Finally, the laser irradiation region can be localized down to a few fractions of a micrometer, which enables organs to be operated on and microsurgery at the subcellular level⁶⁶.

The vast field of laser surgery is summarized briefly here, using ophthalmology as an example (Fig. 5). A continuous wave CO₂-laser radiation can be used to heat the surface layer of the vitreous humour of the eye from the ambient temperature to the gel liquefaction temperature and then to aspirate the liquefied area. In this case, the energy consumed in liquefaction (53 cal g⁻¹) is about one-tenth of that required for evaporation (576 cal g⁻¹). By increasing the radiated power, intense heating and coagulation of the irradiated region can be achieved. This is used, for example, in the treatment of proliferative diabetic retinopathy by photocoagulation⁶⁷. Focused laser radiation can be used to heat intensely and evaporate small areas inside the eye to open up a pathway into Schlemm's canal in treating glaucoma⁶⁸. In such microsurgical operations, it is essential that photodamage is strictly localized, to avoid intense heating and damage to the surrounding tissues. In this case it is advantageous to use a short, powerful laser pulse which, because of the nonlinear effect occurring at the focal point, can be absorbed even in a transparent tissue. This causes intense local heating and destruction in the area affected by the shock wave resulting from high hydrodynamic pressures; the surrounding tissue suffers no appreciable heating⁶⁵.

The field of application for laser-surgery techniques has been extended with the development of methods to deliver a surgical radiation dose (from a few watts to a few tens of watts) through an optical fibre passed through blood vessels to internal areas of the human body. Laser-angioplasty techniques^{69,70}, in which a fibre optics catheter is used to deliver a laser radiation to atherosclerotic obstructions (plaques) are particularly promising. To evaporate a plaque, it is sufficient to irradiate it by an argon laser of 3–4 W in power output for a few seconds, and the evaporation of a calciferous plaque requires 30–40 s of irradiation with the same laser. The destruction of an obstruction using infrared or visible CW radiation occurs according to a thermal mechanism, and to prevent thermal damage to the vessel walls, one is restricted to piercing only a small-diameter passage. Of interest, therefore, is the change from thermal destruction with CW laser radiation to photochemical ablation of plaques by pulsed ultraviolet radiation. In such photochemical actions, the radiation energy is expended directly in the breakdown of chemical bonds, followed by the destruction of the irradiated material as a result of volatile products⁷¹. Some preliminary reports on the effect of ultraviolet-laser radiation on atherosclerotic plaques *in vitro* show that the mechanism of action here is distinct from the thermal mechanism in visible and infrared irradiation⁷². This conclusion is in line with the results of studying the mechanism and products of plaques destruction by ArF ($\lambda = 193$ nm)⁷³, XeCl ($\lambda = 308$ nm) excimer and other lasers⁷⁴. The specific photo-ablation energy amounts to around 250 cal g⁻¹, that is, it is less than the specific thermodestruction energy⁷⁴. Thus, another promising method can be added to the list of potential photodestruction techniques in laser surgery.

Finally, sharp focusing of laser radiation (microbeam techniques) makes it possible to alter selectively some part of a subcellular organelle in a single living cell⁷⁵. The region of such alterations can be only a quarter of a micrometre in length. The laser wavelength and pulse duration can be varied over a broad range, allowing for photodestruction conditions, from thermal to multiquantum photochemical. This opens up new possibilities

in cell and developmental biology, enabling chromosome microsurgery, surgery of mitotic organelles and cytoplasm⁷⁵, for example. The capabilities of the technique extend into genetic engineering, as it provides experimentalists with a laser scalpel enabling a submicron hole to be pierced in the wall of a cell. The desired gene from the surrounding solution can enter the cell through this hole which is then healed again in a few fractions of a second⁷⁶ (J. Wolfrum, personal communication).

Thus, laser biology and medicine now use all the characteristics of laser light as well as all types of its interaction with biological structures. The many fascinating and rapidly developing laser applications are sure to become more widespread and diverse in the future.

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ARTICLE

Loss of heterozygosity in three embryonal tumours suggests a common pathogenetic mechanism

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Children with the Beckwith-Wiedemann syndrome have a greatly increased potential for the specific development of the embryonal tumours hepatoblastoma, rhabdomyosarcoma and Wilms' tumour. Data obtained with molecular probes suggest that the association between these disparate, rare tumour types reflects a common pathogenetic mechanism that entails the somatic development of homozygosity for a mutant allele at a locus on human chromosome 11.

EMBRYONIC tumours in children may result from a developmental disturbance during organogenesis that causes arrest at an early stage in the normal differentiation process to adult tissue¹. This embryonal tissue continues to proliferate inappropriately at this early stage and produces a mass of immature tissue that has been simultaneously termed an embryonal tumour and an organismal malformation². Thus, these tumours can reasonably be considered to comprise one class of congenital malformations. The high-frequency association among some of these malformations has allowed clinical classifications of the developmental anomaly syndromes³. One example of this clinical grouping is the autosomal dominant Beckwith-Wiedemann syndrome (BWS)^{4,5}, which is composed of multiple congenital malformations coupled with a concurrent high risk for the development of specific rare childhood tumours. More than 10% of all individuals with BWS will develop one of the specific oncogenic malformations, which include Wilms' tumour, hepatoblastoma, rhabdomyosarcoma and adrenal carcinoma, in association with the growth excess disorders that characterize the syndrome^{5,6}. Even those individuals with the incomplete, partially penetrant form of the syndrome who display only a subset of these physical anomalies have increased risk for the development of specific malignancies⁶. In fact, these patients may develop tumours of more than one type simultaneously, such as the appearance of Wilms' tumour with adrenal carcinoma⁷ or adrenal adenoma⁸. Similar simultaneous development of more than one tumour type in patients with no readily apparent organismal anomalies has also been described. These include the development of Wilms' tumour with rhabdomyosarcoma⁹ or hepatoblastoma¹⁰, hepatoblastoma with adrenal adenoma¹¹, and rhabdomyosarcoma with adrenal carcinoma¹².

The suggestion of the ontogenetic relatedness of these tumour types is also supported by histopathological analyses. The embryonal tissue from which Wilms' tumour seems to arise

consists of the metanephric blastema that has failed to undergo the normal maturation process¹³. A common aetiology for Wilms' tumour, hepatoblastoma and rhabdomyosarcoma is suggested by the ability of these embryonal tumours to undergo divergent differentiation to form a tissue that does not occur in the normal end-stage organ. For example, fetal rhabdomyomatous nephroblastoma is a variant Wilms' tumour comprising striated muscle of the kidney¹⁴. The rhabdomyoblasts appear to arise by divergent or aberrant differentiation from the metanephric blastema¹⁴. Rhabdomyomatous tissue has also been observed in some hepatoblastomas¹⁵. Additionally, heterotropic primary tumours apparently consisting of adrenal carcinoma and rhabdomyosarcoma have been observed in surgically excised livers^{16,17}.

It has been reported that the expression of recessive mutant alleles at the *WAGR* locus on human chromosome 11 band p13 and the *Rb-1* locus on human chromosome 13 band q14 are involved in the development of Wilms' tumour¹⁸⁻²¹ and retinoblastoma²², respectively. Expression of the initial predisposing recessive mutation at these loci is effected by abnormal somatic chromosomal segregation events whose net result is the complete loss of the dominant balancing wild-type allele. The mitotic mechanisms that accomplish this removal included nondisjunctional loss and mitotic recombination. The results of these mitotic events are consistent with the production of a cell homozygous for the mutant allele at the retinoblastoma or Wilms' tumour locus. Evidence in support of this interpretation was recently obtained by the demonstration that the chromosome 13 homologue remaining in familial retinoblastoma tumours was always derived from the affected parent²³.

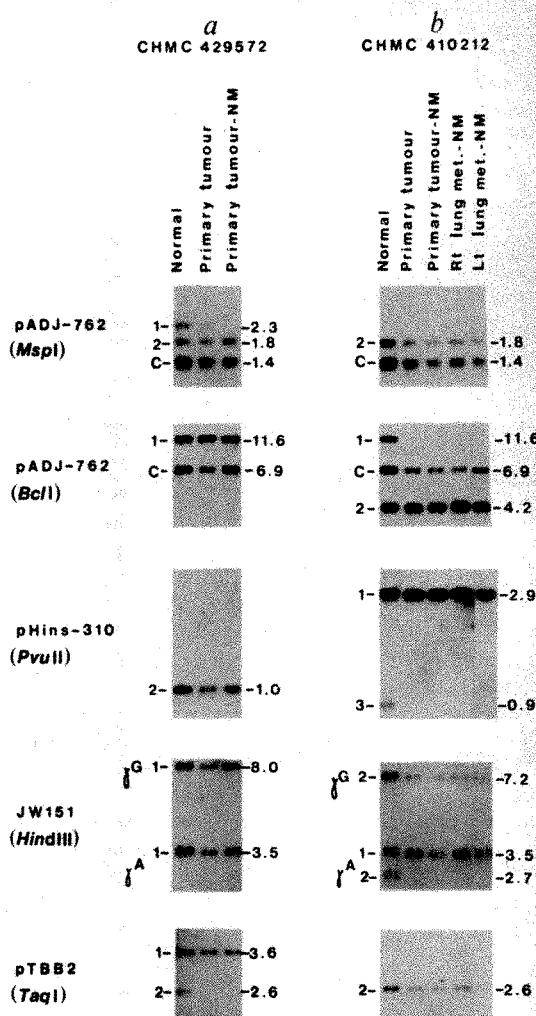
The clinical association of Wilms' tumour with other specific rare tumours in the presence of the growth excess syndromes, the development of more than one rare tumour in the same individual, and the presence of heterotropic tissue in these tumours could be simply circumstantial. Alternatively, these clinical associations may reflect a common aetiological event so that each of the developmental anomalies, including the tumours, arises after mutation of the same locus. Such mutations

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Fig. 1 Loss of constitutional heterozygosity at loci on human chromosome 11p in hepatoblastoma; *a*, patient A; *b*, patient B.

Methods. DNA was isolated from normal tissue (peripheral leukocytes), surgical specimens of primary tumour or from a xenograft of the primary tumour passed twice in immunodeficient nude mice (NM), as described previously^{18,22,26}. DNA was also isolated from xenograft tumours derived from right and left lung metastases (passages 3 and 1, respectively) removed from patient B following thorotomy and wedge resection to remove metastatic hepatoblastoma nodules. DNA from each of these tissues was digested to completion with the indicated restriction endonucleases, subjected to agarose gel electrophoresis and transferred to Zeta-por membranes (AMF-Cuno) in 1.5 M NaCl/0.15 M sodium citrate. The membranes were prehybridized at 42 °C for 16 h in 0.75 M NaCl/0.075 M sodium citrate, 0.2% Ficoll 400, 0.2% bovine serum albumin (BSA), 0.2% polyvinylpyrrolidone, 50 mM sodium phosphate pH 6.7, 100 µg ml⁻¹ denatured salmon-sperm DNA and 50% formamide. The samples were hybridized to four recombinant DNA probes physically homologous to loci unique to the subregion p15 of the short arm of human chromosome 11 (ref. 34), reveal restriction fragment length polymorphisms and exhibit close genetic linkage³¹. The probe pHins-310, homologous to the insulin gene³⁵, and the probe pTBB-2, homologous to the c-Ha-ras-1 gene³⁶, exhibit polymorphic alleles of various lengths, generated by insertions or deletions of DNA sequences, in *PvuII*- and *TaqI*-digested DNA, respectively³⁴⁻³⁶. The probe JW151 is homologous to the β -globin gene cluster³⁷ and reveals restriction fragment length alleles of the γ^G portion of the locus of 8.0 and 7.2 kilobases (kb) and alleles of 3.5 and 2.7 kb at the γ^A portion of the locus in *HindIII*-digested DNA³⁷. The probe pADJ-762, an arbitrary genomic DNA segment, reveals alleles of 2.3 and 1.8 kb in *MspI*-digested DNA and of 11.6 and 4.2 kb, as well as a constant 6.9 kb band, in *BclI*-digested DNA³³. The ³²P-labelled nick-translated probes were hybridized to the membranes at 47 °C for 24 h in 0.75 M NaCl/0.075 M sodium citrate, 0.02% Ficoll 400, 0.02% BSA, 0.02% polyvinylpyrrolidone, 20 mM sodium phosphate pH 6.7, 100 µg ml⁻¹ denatured salmon-sperm DNA, 10% dextran sulphate and 50% formamide. The membranes were washed once at room temperature in 0.3 M NaCl/0.03 M sodium citrate/0.1% SDS for 30 min and then twice for 30 min each at 65 °C in 0.015 M NaCl/0.0015 M sodium citrate/0.1% SDS. The membranes were then exposed to Kodak XAR-5 X-ray film backed with Dupont lightning-plus intensifying screens for 1-3 days. Alleles at each locus are designated 1 or 2 according to decreasing length; C indicates a common, invariant band. Numbers to the right of each autoradiograph indicate the molecular size of the indicated allele in kilobases, derived from the standards run with each gel.



could be revealed by mitotic segregation events, similar to those demonstrated previously for Wilms' tumour¹⁸⁻²¹ and retinoblastoma²², which would serve specifically to produce hepatoblastoma, rhabdomyosarcoma and adrenal carcinoma tumours that have lost constitutional heterozygosity. In particular, if such homozygosity was specifically obtained for chromosome 11p, the data would imply that the Wilms' tumour locus plays a pleiotropic role in developing embryonic kidney, liver, adrenal and muscle tissue, perhaps by differential stage-specific expression. Such data would also indicate a limited number of genetic loci in which recessive mutations play a role in tumour development, each with specificity for a larger subset of tissue types. To distinguish between these hypotheses, we performed studies similar to those described previously for retinoblastoma²² and Wilms' tumour¹⁸⁻²¹.

Hepatoblastoma development

Figure 1 displays the constitutional and tumour genotypes at loci on chromosome 11p for two hepatoblastoma patients. CHMC 429572 (Patient A) was diagnosed with embryonal hepatoblastoma at 6 years of age. He was constitutionally heterozygous (that is, displayed both parental fragment length alleles) at the two loci defined by pADJ-762 in *MspI*-digested DNA and pTBB-2 in *TaqI*-digested DNA. One of the alleles at each of these loci was missing in the primary hepatoblastoma biopsy tissue and in the first passage of the biopsy in an immunodeficient mouse (Fig. 1a). CHMC 410212 (Patient B) was diagnosed with embryonal hepatoblastoma at 1.2 years of age. She was constitutionally heterozygous at the three loci on

human chromosome 11p defined by pADJ-762 in *BclI*-digested DNA, pHins-310 in *PvuII*-digested DNA and the γ^A portion of the β -globin locus defined by JW151 in *HindIII*-digested DNA. Each of these loci had become homozygous or hemizygous in tissue obtained as a primary hepatoblastoma biopsy, in the primary tumour tissue transplanted twice in immunodeficient mice and in metastatic tumours from the right and left lungs of the same patient (Fig. 1b). A third patient, CHMC 304772, was diagnosed with hepatoblastoma with elements of hepatocellular carcinoma at 3.1 years of age. He was constitutionally heterozygous at the two loci defined by pADJ-762 in *MspI*-digested DNA and pTBB-2 in *TaqI*-digested DNA; both alleles at each of these loci were apparent in the tumour (data not shown).

These results are similar to those reported for Wilms' tumour¹⁸⁻²¹. The somatic loss of constitutional heterozygosity in the two hepatoblastoma tumours shown in Fig. 1 could be effected by abnormal mitotic events. Mechanisms that have been demonstrated include the non-disjunctional loss of the wild-type chromosome with reduplication of the mutant chromosome, non-disjunctional loss of the entire wild-type chromosome and deletion or loss of the region of the chromosome containing the marker loci¹⁸⁻²². To distinguish between these possibilities, DNA samples from the normal tissues and primary tumour biopsies described in Fig. 1 were hybridized concurrently with a DNA probe homologous to loci on chromosome 11 (pADJ-762) and chromosome 17 (DOSLC4) (ref. 24). The resultant hybridization signals were analysed by quantitative densitometry (Table 1). The peak area obtained with each signal was calculated and the ratios of the signal unique to chromosome 11 to that unique to chromosome 17 were compared between the normal and tumour

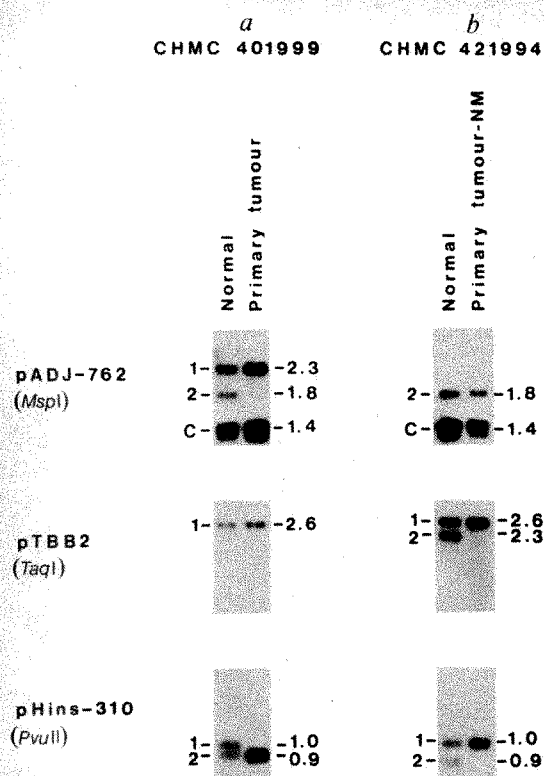


Fig. 2 Loss of constitutional heterozygosity at loci on human chromosome 11p in rhabdomyosarcoma; *a*, patient A; *b*, patient B. **Methods.** *a*, Normal-tissue DNA was isolated from peripheral leukocytes and tumour DNA isolated directly from the primary tumour specimen obtained at surgery. *b*, Normal-tissue DNA was isolated from peripheral leukocytes; tumour DNA was isolated from a first passage xenograft of the primary tumour (NM). The conditions for digestion of the DNA samples, electrophoresis, Southern transfer to nylon membranes, hybridization and autoradiography were as described previously^{18,22,26} and are detailed in Fig. 1 legend. Alleles at each locus are designated 1 or 2 according to decreasing length; C indicates a common, invariant band. Numbers to the right of each autoradiograph indicate the molecular size of the indicated allele in kilobases, derived from standards run with each gel.

tissues. In each case, these ratios were similar in the two tissues, indicating that the loci on chromosomes 11 and 17 were present in both normal and tumour tissue in the same copy number. These data indicate that the tumour specific losses of constitutional heterozygosity described for the hepatoblastomas in Fig. 1 were effected by non-disjunctional loss of the wild-type chromosome with reduplication of the mutant chromosome rather than by chromosomal loss or deletion. It is also possible that loss of heterozygosity at the loci tested here, all of which map to the telomeric portion of chromosome 11, could arise by mitotic recombination between the centromere and the marker loci. We are not presently able to distinguish between the recombination and loss/reduplication mechanisms; such distinctions await the development of probes homologous with loci between the mutant hepatoblastoma locus and the centromere.

Rhabdomyosarcoma development

Figure 2 displays constitutional and tumour genotypes of two rhabdomyosarcoma patients. CHMC 401999 (Patient A) was diagnosed with embryonal rhabdomyosarcoma at 0.8 years of age. She was constitutionally heterozygous at the two loci defined by pADJ-762 in *MspI*-digested DNA and pHins-310 in *PvuII*-digested DNA. CHMC 421994 (Patient B) was diagnosed with embryonal rhabdomyosarcoma at 1.1 years of age. She was constitutionally heterozygous at the loci homologous to pTBB-2 in *TaqI*-digested DNA and pHins-310 in *PvuII*-digested DNA.

Table 1 Densitometric dosage analysis of DNA from normal leukocytes and primary tumour biopsies from hepatoblastoma and rhabdomyosarcoma patients

Patient	Tissue	Chromosome 11 chromosome 17	Tumour normal
CHMC 429572	Leukocytes	2.84	1.12
	Hepatoblastoma	3.20	
CHMC 410212	Leukocytes	1.16	1.03
	Hepatoblastoma	1.20	
CHMC 401999	Leukocytes	2.24	1.06
	Rhabdomyosarcoma	2.38	
CHMC 421994	Leukocytes	3.15	0.93
	Rhabdomyosarcoma	2.92	

Normal and tumour DNA from the patients described in Figs 1 and 2 were digested with the restriction endonuclease *BclI*, transferred to nylon membranes and hybridized simultaneously to the radiolabelled probes pADJ-762 and DOSLC4, as described in Fig. 1 legend. These probes are homologous to unique loci on human chromosomes 11 (ref. 33) and 17 (ref. 24), respectively. The resultant autoradiogram was analysed by scanning densitometry and the peak areas corresponding to each hybridization signal were calculated by electronic integration. The peak areas specific to chromosomes 11 and 17 were compared in each sample and these normalized values were compared for each tumour/normal tissue pair.

Only one of the two codominant alleles at each of these loci was apparent in the primary tumour biopsy from Patient A and in the first-passage xenograft of the primary rhabdomyosarcoma from Patient B. Patient CHMC 405680 was diagnosed with alveolar rhabdomyosarcoma at 3.3 years of age. He was constitutionally heterozygous at the two loci on chromosome 11p15 defined by pADJ-762 in *BclI*-digested DNA and pTBB-2 in *TaqI*-digested DNA; heterozygosity was maintained at both loci in the corresponding tumour sample (data not shown). DNA isolated from normal and tumour samples from patients A and B was hybridized to DNA probes homologous to loci on chromosome 11 (pADJ-762) or chromosome 17 (DOSLC4). Densitometric analysis of the resultant autoradiographic signals, as described above for the hepatoblastoma cases, is shown in Table 1. Again, the number of copies of the two loci was similar in the normal and tumour tissues, indicating that the loss of constitutional heterozygosity in the tumours was effected by a mitotic mechanism which resulted in loss of the wild-type chromosome 11 homologue with maintenance and reduplication of the mutant chromosome, rather than by simple chromosomal loss or deletion of the segment of the chromosome containing the marker loci.

Chromosomal specificity

The somatic losses of constitutional heterozygosity described above for hepatoblastoma and rhabdomyosarcoma, and previously for Wilms' tumour¹⁸⁻²¹, could serve specifically to unmask initial predisposing mutations on chromosome 11 as shown previously for chromosome 13 in retinoblastoma^{22,23}. Alternatively, the attainment of chromosome 11 homozygosity in these tumours might represent a portion of a generalized loss of heterozygosity, such as that proposed based on analyses of isozymic alleles²⁵. To distinguish between these possibilities, the Southern transfers shown in Figs 1 and 2 were treated with alkali to remove the chromosome 11 probes²⁶ and then hybridized to several probes that reveal restriction site polymorphism on other human autosomes^{24,26-28}. In every case where constitutional heterozygosity was present, both codominant alleles could be distinguished in the corresponding tumour tissue (Table 2). Thus, the tumour from patient CHMC 429572 maintained heterozygosity at loci mapped to chromosomes 13, 14 and 22; the tumour from patient CHMC 410212 for loci mapped to chromosomes 6 and 13; and the tumours from patients CHMC 401999 and CHMC 421994 for loci mapped to chromosomes 13 and 20. In total, these tumours, which had become homozygous at several loci on chromosome 11, retained heterozygosity at other loci on chromosomes 6, 13, 14, 20 and 22. These data indicate that the losses of constitutional heterozygosity for chromosome 11 in these tumours are specific events rather than part of a generalized loss during tumorigenesis. The maintenance

Table 2 Alleles present at loci on chromosomes other than 11 in normal and tumour tissue from hepatoblastoma and rhabdomyosarcoma cases

Probe	Enzyme	Chromosome	Alleles present											
			Hepatoblastoma								Rhabdomyosarcoma			
			CHMC 429572			CHMC 410212					CHMC 401999		CHMC 421994	
			N	T ¹	T ²	N	T ³	T ⁴	T ⁵	T ⁶	N	T ⁷	N	T ⁸
p3C7	<i>Msp</i> I	6	1,1	1,1	1,1	1,2	1,2	1,2	1,2	1,2	1,1	1,1	2,2	2,2
pL2.56	<i>Hind</i> III	6	1,1	1,1	1,1	1,1	1,1	1,1	1,1	1,1	2,2	2,2	2,2	2,2
p7F12	<i>Msp</i> I	13	ND	ND	ND	ND	ND	ND	ND	ND	1,2	1,2	1,2	1,2
	<i>Taq</i> I	13	1,2	1,2	1,2	2,2	2,2	2,2	2,2	2,2	2,2	2,2	2,2	2,2
p1E8	<i>Msp</i> I	13	1,1	1,1	1,1	1,2	1,2	1,2	1,2	1,2	2,2	2,2	2,2	2,2
p9A7	<i>Hind</i> III	13	1,2	1,2	1,2	1,2	1,2	1,2	1,2	1,2	1,1	1,1	1,2	1,2
pAW101	<i>Eco</i> RI	14	1,2	1,2	1,2	2,2	2,2	2,2	2,2	2,2	ND	ND	ND	ND
DOSLC2	<i>Msp</i> I	20	1,1	1,1	1,1	1,1	1,1	1,1	1,1	1,1	1,2	1,2	1,2	1,2
DOSLC8	<i>Taq</i> I	22	1,2	1,2	1,2	2,2	2,2	2,2	2,2	2,2	2,2	2,2	2,2	2,2

Tumour and normal DNA from hepatoblastoma patients CHMC 429572 and CHMC 410212, as well as rhabdomyosarcoma patients CHMC 401999 and CHMC 421994, were digested with appropriate restriction endonucleases, transferred to nylon membranes, hybridized to the indicated ³²P-labelled probes, washed and exposed to X-ray film as described in Fig. 1 legend. Each of the probes is homologous with a locus on the indicated chromosome^{24,26-28}. Tumour samples were: T¹, primary surgical biopsy; T², first passage xenograft of T¹; T³, primary surgical biopsy; T⁴, second passage xenograft of T³; T⁵, third passage xenograft of metastatic T³ excised from the right lung; T⁶, first passage xenograft of metastatic T³ excised from the left lung; T⁷, primary surgical biopsy; T⁸, first passage xenograft. In all cases, normal tissue was obtained as peripheral leukocytes. N, normal tissue; T, tumour tissue; ND, not determined.

Table 3 Alleles at loci on chromosome 11 in normal and tumour tissues from Ewing's sarcoma and osteogenic sarcoma patients

Patient		Tissue	Alleles present				pTBB3
			pADJ-762	JW151			
				<i>Msp</i> I	<i>Bcl</i> I	γ^G - <i>Hind</i> III	
A Ewing's sarcoma							
1	CHMC 319634	N	2,2	1,2	2,2	1,2	1,2
		T	2,2	1,2	2,2	1,2	1,2
2	CHMC 414222	N	2,2	1,2	2,2	1,1	1,2
		T	2,2	1,2	2,2	1,1	1,2
3	CHMC 434672	N	2,2	1,2	1,2	1,1	1,1
		T	2,2	1,2	1,2	1,1	1,1
B Osteogenic sarcoma							
1	CHMC 438160	N	2,2	ND	1,1	1,2	1,2
		T	2,2	ND	1,1	1,2	1,2
2	CHMC 428687	N	2,3	ND	2,2	1,2	1,2
		T	2,3	ND	2,2	1,2	1,2
3	CHMC 434642	N	2,2	ND	2,2	1,2	1,1
		T	2,2	ND	2,2	1,2	1,1

Tumour and normal DNA from Ewing's sarcoma patients CHMC 319634, CHMC 414222 and CHMC 434672, as well as osteogenic sarcoma patients CHMC 438160, CHMC 428687 and CHMC 434642, were digested with the indicated restriction endonucleases, transferred to nylon membranes, hybridized to the indicated radiolabelled probes, washed and exposed to X-ray film as described in Fig. 1 legend. Tumour (T) samples were xenografts of primary surgical biopsies except in the case of CHMC 434642 where primary surgical biopsy material was obtained. Normal (N) tissue was obtained as peripheral blood leukocytes. ND, not determined.

of heterozygosity in these tumours for loci mapped to chromosome 13 is of particular interest because the same markers have previously allowed detection of retinoblastoma-specific losses of constitutional heterozygosity^{22,23}. The data described in Table 2 also provide assurance that the tumour-specific differences from constitutional genotypes shown in Figs 1 and 2 do not arise from simple mispairing of the samples. In fact, we have examined these samples at many more non-chromosome 11 loci than those shown in Table 2 and we have not observed any instance in which the homozygous alleles in each pair of tissues did not precisely match.

Tumour specificity

The previously described clinical data, as well as the specific loss of heterozygosity for loci on human chromosome 11 during the genesis of rhabdomyosarcoma and hepatoblastoma (described above) and Wilms' tumour¹⁸⁻²¹, suggest a pathogenetic mechanism shared by these three clinically associated tumour types. To determine whether these events are com-

mon among more than these three childhood cancers, we performed the analyses described in Table 3. We isolated DNA from normal and tumour samples obtained from three children each with Ewing's and osteogenic sarcoma. These DNA samples were then examined with the probes homologous to loci on chromosome 11, exactly as described in Figs 1 and 2. In each case, constitutional heterozygosity was maintained in these tumours. It has been reported previously²² that another pediatric tumour, retinoblastoma, maintained chromosome 11 heterozygosity in several tumour samples. These results suggest that the loss of constitutional heterozygosity in rhabdomyosarcoma, hepatoblastoma and Wilms' tumour is specific to chromosome 11 and also displays specificity in those childhood tumours in which it is a pathogenetic mechanism.

Discussion

Here, we have described molecular genetic evidence suggesting that human embryonal tumours reflect abnormalities in genes important to normal human organismal development. These

data unite two widely disparate classes of observation: the high-frequency clinical associations between rare tumours and congenital malformation syndromes³; and the specific somatic loss of constitutional heterozygosity in the oncogenesis of embryonal cancers¹⁸⁻²².

Our data suggest a common chromosomal pathogenetic mechanism in rhabdomyosarcomas and hepatoblastomas similar to that previously shown in Wilms' tumours¹⁸⁻²¹. These results imply a common aetiology in the genesis of these three embryonal tumours that involves pleiotropic expression of a mutant allele at the Wilms' tumour (*WAGR*) locus on human chromosome 11 band p13 (ref. 29). Chromosomal mechanisms that serve to unmask these recessive mutant alleles by elimination of the balancing wild-type homologue occurring at times specific to the development of kidney, liver or striated muscle could then result in an embryonal tumour specific to the developing tissue of the organ. Pathological observations showing that embryonal tumours maintain immature differentiation states imply that the product of the *WAGR* locus is necessary for the ordered progression of normal differentiation to occur. Failure to produce the gene product would then result in the persistence of precursor embryonal cells of each organ and thereby account for persistent renal blastema or nephroblastomatosis. The loss of function effected by aberrant chromosomal events at the various times in organismal development might explain the aberrant differentiation that is observed as heteroplasia^{14,15} in these tumours and that leads to the previously unexplainable presence of striated muscle precursors, rhabdomyoblasts, in embryonal tumours of the kidney (Wilms' tumour) and the liver (hepatoblastoma). Thus, several lines of clinical evidence suggest a pathogenetic association between these three embryonal tumours. It is not known whether this association results from differential expression of mutations in the same gene or represents overlapping deficiencies in a larger multigene complex. This distinction cannot be drawn until the gene(s) is isolated and analysed. Clearly, however, the genesis of each of these rare tumours involves the specific loss of constitutional heterozygosity for human chromosome 11 and these events occur in these clinically associated tumours and in none of the other embryonal tumours we have examined. Although we have not yet been able to examine adrenal carcinoma tissues, it is reasonable to propose, based on these findings and clinical observations^{5-8,11,12,17,18}, that this tumour also arises following the attainment of chromosome 11 homozygosity.

Most developmental anomaly syndromes associated with high risk for tumour development segregate in families with the characteristics of a single autosomal dominant mutation³. A genetic component in the aetiology of Beckwith-Wiedemann syndrome has been proposed based on its autosomal dominant mode of inheritance⁴ and by the observation of a constitutional karyotypic abnormality of human chromosome 11p (ref. 30) in

a few patients with the syndrome. These observations, taken together with the evidence reported here, are consistent with the idea that a genetic locus on the short arm of human chromosome 11 is involved in the determination of the phenotypic manifestations of this disorder, including the developmental and oncological malformations. The distinction between these two types of abnormalities may arise by differential requirements for the product of the normal locus at varying times of development. Alternatively, the developmental malformations may represent the heterozygote phenotype, whereas the various tumour types may arise only after the attainment of complete homozygosity for a mutant allele at the locus. Either of these possibilities implies that loci in which defects give rise to heritable groups of developmental disorders are also responsible for the oncological disorders associated with the syndrome.

These data also have broad implications for genetic mapping. They indicate that the number of genetic loci whose recessive mutant alleles predispose to cancer is more limited than might have been suspected based on previous studies¹⁸⁻²². Certainly, it does not now seem as if there is one such locus for each tumour type, but it is likely that these loci will have broader, but specific, tissue preference. Studies to identify loci similar to *Rb-1* and *WAGR* may now be designed more effectively by selecting tumour types based on their clinical associations. This approach has proved to be useful in this study and our preliminary studies show that retinoblastoma and osteosarcoma share both clinical association and a molecular pathogenetic mechanism. Conversely, the identification of which chromosome carries a recessive predisposing mutation by the kinds of comparisons of tumour and constitutional genotypes described here, in tumours which cluster with high frequency in the various developmental anomaly syndromes, may expedite the selection of genetic markers for pedigree analysis^{31,32}. For example, the identification of chromosomes that undergo specific losses of constitutional heterozygosity during oncogenesis of astrocytoma, medullary thyroid carcinoma and medulloblastoma may lead to localization of the genetic locus in their clinically associated autosomal dominant syndromes of neurofibromatosis, multiple endocrine neoplasia and basal cell nevus syndrome, respectively. These sorts of analyses may accelerate the development of recombinant DNA-based premorbid detection of these diseases.

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Crystallographically aligned metal–oxide composite made by reduction of a directionally solidified oxide–oxide eutectic

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Aligned metal–oxide composite materials have been grown from the melt by directional solidification at eutectic composition of several binary metal–oxide systems. The resulting microstructures consist of metallic fibres $\sim 1\ \mu\text{m}$ in diameter imbedded in an oxide matrix^{1,2}. The occurrence of such fibrous structures, as opposed to lamellar structures, corresponds to eutectic compositions for which the volume fraction of the minor component is $<30\%$ (ref. 3). The work reported here presents a different route to the fabrication of aligned metal–oxide composites, making the production of fine-scale lamellar microstructures possible. The method consists of the chemical reduction of lamellar oxide–oxide eutectic structures in conditions of temperature and partial pressure of oxygen in which only one of the oxides is transformed to the corresponding metal. We show here how the method can be successfully applied to the preparation of aligned Ni–ZrO₂ lamellar structures.

Directionally-solidified oxide–oxide eutectics have been extensively studied in the past two decades, experimentation being carried out in more than 20 binary systems⁴. Several of them were examined in our laboratory within the framework of general studies of the structure and properties of oxide interfaces^{5–7}; these systems were chosen with the object of creating interfaces between a sodium chloride type structure (NiO) and other phases of cubic symmetry. The systems which were examined include: NiO–CaO (sodium chloride); NiO–NiAl₂O₄ (spinel); NiO–calcium-stabilized cubic ZrO₂ (fluorite); NiO–Y₂O₃ (fluorite-related).

Well-aligned eutectic microstructures were obtained and growth directions of the oriented structures, crystallographic relations existing between the two phases of each eutectic as well as the indices of the interface planes were determined^{8–10}.

It is of interest to extend these studies to metal–oxide interfaces by trying to reduce one of the oxides of an oxide–oxide eutectic structure into the corresponding metal. We chose the binary system NiO with stabilized cubic ZrO₂ because of the stability of ZrO₂ in conditions in which NiO could be easily

reduced into nickel. Another reason for this choice was the possible fabrication of a fine-scale structure made of alternate lamellae of ionically conducting (ZrO₂) and electronically conducting (Ni) phases.

Directional-solidification experiments in the NiO–ZrO₂ system were carried out in a floating-zone device¹¹. The starting rods which were used had a composition of 70 mol.% NiO, 25.5 mol.% ZrO₂, 4.5 mol.% CaO and were prepared by pressing (1 kbar) a mixture of finely ground 99.999% pure NiO, 99.9% pure ZrO₂ and 99.9% pure CaCO₃. The pressed rods, 12 mm in diameter, were sintered in air at 1,200 °C for 24 h before directional solidification was undertaken.

Growth experiments were performed in air. Optical examination of the microstructures indicate well aligned eutectic lamellar structures, the two phases of the eutectic being pure nickel oxide ($a_{\text{NiO}} = 4.178\ \text{\AA}$) and calcia stabilized cubic zirconia ($a = 5.13\ \text{\AA}$). The variation of the interlamellar spacing λ with the solidification rate R follows the law $\lambda^2 R = \text{constant}$, proposed by Tiller¹² and verified in many eutectic systems. Typically, for a growth rate of $1.5\ \text{cm h}^{-1}$, the interlamellar spacing is $\sim 3\ \mu\text{m}$ (Fig. 1).

Microfragments of the directionally solidified samples oriented along the growth direction were studied by rotating crystal, Weissenberg and Buerger precession methods, to determine preferred growth directions and epitaxial relations. Growth directions are mainly [110] for nickel oxide and [001] for calcia-stabilized zirconia: epitaxial relationships are [110]//[001] and (111)/(100), the interface planes being (111)_{NiO} and (100)_{ZrO₂} (ref. 13).

The information relative to growth directions of the eutectic phases was confirmed by X-ray diffractometry experiments carried out on transverse sections of the samples: the only peaks observed were high-intensity peaks corresponding to planes normal to growth directions, that is (220) for NiO and (200) and (400) for calcia-stabilized zirconia.

Directionally solidified samples, 3-mm thick, were submitted for 70 h to a chemical reduction treatment at 1,075 °C under a CO/CO₂ mixture (CO/CO₂ = 0.1) corresponding to $P_{\text{O}_2} = 1.6 \times 10^{-11}$ atmospheres, that is, conditions where NiO should be totally reduced to nickel¹⁴.

The microstructure resulting from reduction of the samples presented in Fig. 2 indicates that the aligned nature of the original eutectic structure and the cohesion between the two phases are maintained. Powder X-ray diffraction experiments on crushed samples show total reduction of NiO, the only observable lines being those of pure nickel ($a = 3.52\ \text{\AA}$) and of unaltered calcia-stabilized zirconia, the lattice parameter of which is the same as that found in the eutectic structure.

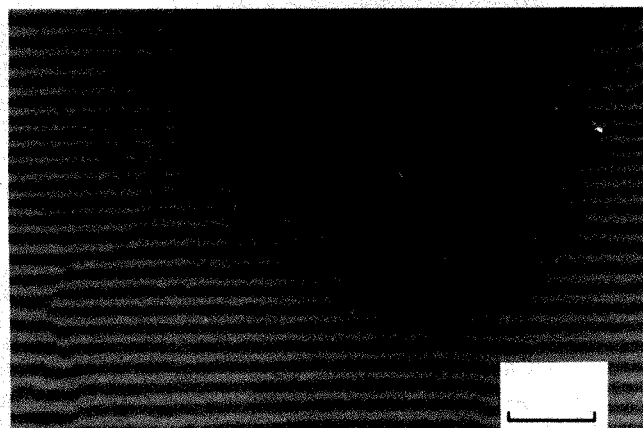


Fig. 1 Transverse section of the nickel oxide–calcium oxide–stabilized zirconia eutectic (70 mol.% NiO, 30% ZrO₂–CaO) solidified at $1.5\ \text{cm h}^{-1}$. Scale bar, $10\ \mu\text{m}$.

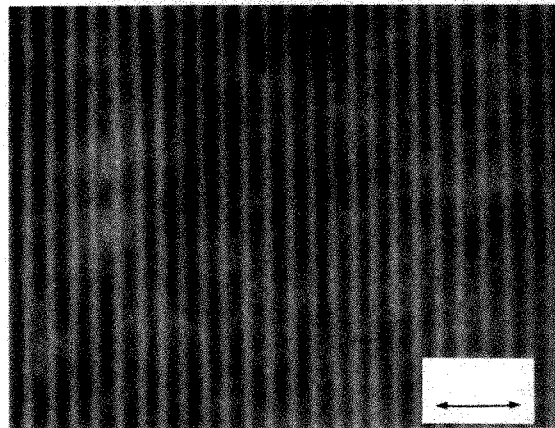


Fig. 2 Crystallographically aligned metal–oxide composite structure made by reduction of a directionally solidified oxide–oxide eutectic. Scale bar, $10\ \mu\text{m}$.

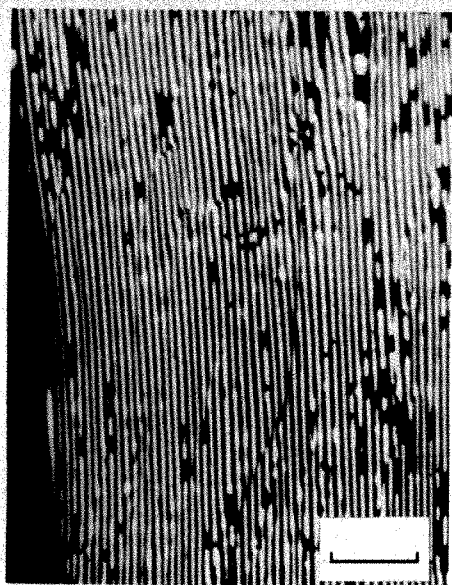


Fig. 3 Characteristic feature of a Ni-ZrO₂ sample showing presence of elongated cavities in the Ni phase. A crack can be seen on the left. Scale bar, 30 μ m.

In some areas of the samples, elongated cavities presumably caused by the elimination of oxygen from the nickel oxide phase can be observed in the nickel phase. A few cracks also occur, the region adjacent to these being depleted in cavities (Fig. 3), indicating cavity coalescence. Observation at higher magnification by scanning electron microscopy confirms these features and particularly the boundary coherence. A change in lamellar spacing on reduction was not detectable. This can be explained by the fact that despite the volume change when transforming from NiO to Ni, the resulting modification of lamellar spacing is theoretically small enough to lie within experimental error of the measurements.

X-ray diffractometry experiments performed on transverse sections of samples of oxide-oxide eutectic structure submitted to reduction by CO/CO₂ mixtures show, compared with the powder diffractograms of the same material, very strong (220) nickel and (200) and (400) zirconia peaks (Fig. 4).

Considering the above results concerning the orientation of the two phases of the NiO-ZrO₂ samples, it seems that the oriented nature of the ZrO₂ phase remains unchanged; the nickel phase seems also to be perfectly oriented, the (110) orientation of the nickel lamellae being that of the original nickel oxide phase. Taking into account the f.c.c. structure of nickel and the NaCl-type structure of nickel oxide, it appears that the reduction takes place by a topotactical process in which the departure of oxygen from the NiO phase leaves intact the f.c.c. nickel sublattice which contracts, preserving the monocrystalline nature of the nickel-based phase. This mechanism was confirmed by separate X-ray diffractometry experiments in which large monocrystalline NiO samples which were chemically reduced in the conditions described above showed transformation into mostly monocrystalline nickel.

These results may be compared with those obtained by Little *et al.*¹⁵ who examined by transmission electron microscopy (TEM) the early stages of low-temperature (200 °C) reduction of nickel oxide and observed the formation of both epitaxial and non-epitaxial nickel nuclei, the latter being limited in number at the beginning of the process but becoming predominant as reduction time progressed. Little *et al.* concluded that loss of epitaxy is a feature of the growth of the nuclei. The presence in our experiments of non-epitaxial nickel, if any, was minor, as indicated by Fig. 4b. One can rationalize the difference in behaviour observed in the two sets of experiments by the large temperature gap which separates them. At higher temperature, more nucleation would lead to small epitaxial nuclei which would rapidly coalesce into larger grains of quasi-iden-

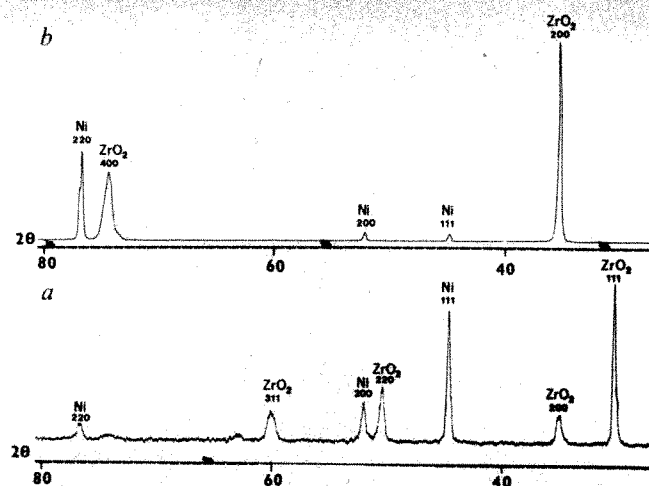


Fig. 4 X-ray diffractometry of the material resulting from reduction of eutectic NiO-ZrO₂: a, powder pattern; b, transverse section of eutectic structure.

tical orientation. An additional reason for the retention of the nickel oxide orientation in nickel may be found in the crystallographic constraint provided by the ZrO₂ phase.

We have shown, therefore, that reduction of lamellar oxide-oxide eutectics may lead to fine-scale alternate metal-oxide structures. The preliminary X-ray studies indicate that, at least in the system under examination, the crystallographic relationships between the two phases of the original structure remain unchanged after reduction of one of the oxides. More elaborate X-ray and TEM studies are being performed to identify finally the Miller indices of the interface planes which apparently also remain unchanged, and second to better describe the Ni-ZrO₂ interface.

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Continental volume and freeboard through geological time

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Approximate constancy of continental freeboard since the Archaean¹⁻³ has been used to argue that no growth of the continental crust occurred during the Proterozoic and Phanerozoic^{4,5} and that there was limited vertical movement of old cratons.⁶ However, it has been shown^{7,8} that constancy of freeboard implies net growth of the continental crust, because the ocean basins deepen as the heat flow from the mantle decreases with time (crustal thickness d_c , the volume of the oceans V_o and the Earth's surface area A_e being constant). A net growth of about 25% or 1 km³ yr⁻¹ is necessary to maintain freeboard at a constant value⁷. We further explore here the consequences of approximate constant freeboard for continental growth using a model that relates the volumes of

isostatically compensated continents and oceans to the secular decline in terrestrial heat flow. We find that a post-Archaeon increase in freeboard by 200 m requires continental growth of only 10%, while a decrease in freeboard by 200 m during this same period necessitates a crustal growth of 40%. Shrinkage of the continental crust since the end of the Archaeon can be ruled out. Changes of >10% in post-Archaeon crustal thickness are highly unlikely.

Temporal variations in freeboard (transgressions and regressions of sea level) can arise from melting of polar ice, post-glacial rebound, changes in the number and areal distribution of continental segments⁹, and changes in the volume of mid-ocean ridges and continents. We consider only the secular changes in freeboard due to changes in the area and thickness of the continents, and changes in the volume of the oceans particularly as a consequence of the deepening of the ocean basins with time on a cooling Earth.

There is an uncertainty of a few hundred meters in the difference between freeboard during the early Proterozoic and the present, mainly due to our inability to calibrate the significance or representativeness of present-day freeboard. The uncertainty in how freeboard has changed since the Archaeon, although small, has important consequences for crustal growth.

Continental crustal volume V_c is the product of its area A_c and thickness d_c . Ocean volume V_o is given by $V_o = A_o(d_a + d_b)$, where A_o is the area of the oceans, d_b is the mean depth below the level of the mid-ocean ridges (MOR) and d_a is the depth below sea level of the rise crests (Fig. 1). The areas of the oceans

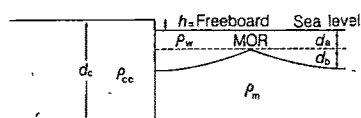


Fig. 1 Sketch of the model. MOR, Mid-ocean ridge; ρ_m , mantle density; ρ_{cc} , crustal density; ρ_w , water density; h , continental freeboard.

and continents sum to the total surface area of the Earth. We assume that A_c has remained constant throughout geological time (the change in A_c due to whole Earth cooling is negligible); V_o is also taken to be constant since the end of the Archaeon. This assumes that all the water in the present oceans outgassed from the interior at the earliest stages of the Earth's evolution and that the amount of water in the form of ice and vapour was not substantially different 2,500-Myr ago from the amount at present.

Continents and oceans are assumed to have always been in isostatic balance. Application of the principle of isostasy¹⁰ to the model of Fig. 1 gives (see Fig. 1 for parameter definitions)

$$d_a - d_a^* = (d_c - d_c^*) \frac{(\rho_m - \rho_{cc})}{(\rho_m - \rho_w)} - (h - h^*) \frac{(\rho_m)}{(\rho_m - \rho_w)} \quad (1)$$

An asterisk indicates present-day values. Ocean-continent density differences extending to great depth in the mantle and varying through geological time could modify the isostatic balance. The connection¹⁰ between d_b and the mean oceanic surface heat flow q_s is

$$\frac{d_b}{d_b^*} = \frac{q_s^*}{q_s} \quad (2)$$

It is generally accepted that q_s has decreased monotonically through geological time through the gradual decline in radiogenic heating and the secular cooling of the Earth¹¹. The decrease tends to follow the decline in internal radioactive heat sources¹¹ and we have adopted a model¹⁰ of the decrease in heat production to derive the form of $q_s(t)$ shown in Fig. 2. Mean oceanic heat flow decreases by about a factor of 4 since 4,500 Myr ago; the decline since the end of the Archaeon is by a factor of ~2. We have ignored the small amount of mantle heat that escapes through the continents consistent with our simplified model and

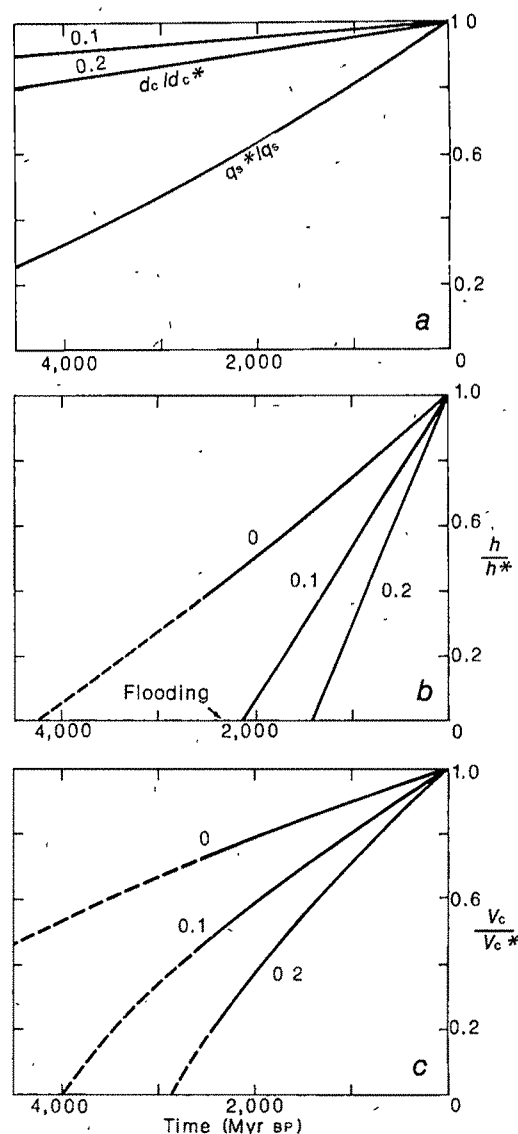


Fig. 2 a, Assumed variations of crustal thickness and mean oceanic heat flow through geological time. b, Continental volume (as a percentage of present volume V_c^*) versus time before present for different assumed crustal thickness variations. c, Freeboard (as a percentage of its present value h^*) as a function of time for V_c and d_c constant. The parameter labelling the curves in b and c is the fractional increase in crustal thickness over 4,500 Myr. The dashed portions of the curves refer to the time period in Earth history when freeboard and crustal thickness are highly uncertain. If h is negative, the continents are flooded. Thus the intersection of the curves in c with the horizontal axis gives the time of emergence of the continents above sea level.

the large uncertainty in past surface heat flow. Figure 2 also shows two models of crustal thickening that we consider; the values 0.1 and 0.2 refer to thickness increases of 10- and 20% over geological time.

Our model relating crustal growth and continental freeboard is similar in many respects to the one studied by Wise¹. Our principal contribution is the addition of equation (2) and the recognition that the cooling of the Earth deepens the ocean basins and thereby has a major role in controlling freeboard.

For constant freeboard and crustal thickness

$$\frac{V_c}{V_c^*} = \frac{A_c}{A_c^*} \frac{(V_o/A_c^*)}{d_a^* + d_b^*(q_s^*/q_s)} \quad (3)$$

in agreement with our previous result⁷. The increase of V_c with time as a consequence of the decline in q_s is readily deduced from equation (3); $V_c(t)$ is shown in Fig. 3. Parameter values are summarized in Table 1; q_s^*/q_s is given in Fig. 2a. The curve labelled 0 in Fig. 2b is for $d_c = \text{constant}$ and shows that a 25%

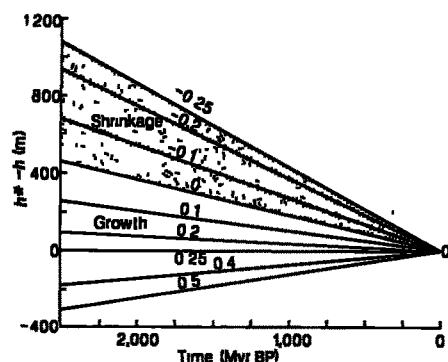


Fig. 3 Freeboard variation with time during the Proterozoic-Phanerozoic for prescribed percentage changes in continental volume between 2,500 Myr ago and the present. Both crustal growth and shrinkage are considered. Crustal thickness is assumed to be constant.

post-Archaeon increase in crustal volume is necessary to maintain constant freeboard⁷.

Since constant freeboard requires 25% post-Archaeon crustal growth (with d_c constant), it is important to determine what change in freeboard would occur for V_c = constant. With both V_c and d_c constant,

$$h^* - h = \left(1 - \frac{\rho_w}{\rho_m}\right) \left(1 - \frac{q_s^*}{q_s}\right) d_c^* \quad (4)$$

Equation (4) shows that freeboard must have been smaller in the past. The decrease in q_s and the deepening of the ocean basins causes a secular regression in sea level, that is, an increase in freeboard. Figure 2c illustrates the variation in freeboard with time computed from equation (4) and the parameter values given in Table 1. At the end of the Archaeon, h is predicted to be only about 40% of its present value (curve 0); freeboard must have increased by 400 m. This seems inconsistent with the geological constraint of approximate constant freeboard, but it is difficult to estimate the uncertainty in this constraint quantitatively.

It is clear from the above that freeboard variations of only several hundred metres are associated with crustal volume variations of tens of per cent. Since freeboard changes of this magnitude cannot be ruled out on geological grounds we consider the general relation between h and V_c for constant d_c .

$$h - h^* = \frac{(\rho_m - \rho_w)}{(\rho_m)} \left[d_c^* + d_b^* \frac{q_s^*}{q_s} - \frac{V_0}{(A_c - V_0/d_c^*)} \right] \quad (5)$$

We have evaluated equation (5) for prescribed changes in continental volume between the end of the Archaeon and the present and show the results in Fig. 3.

As we have already seen, no change in freeboard implies crustal growth of 25% during the post-Archaeon. If freeboard has been within ± 200 m of its present value since the end of the Archaeon, then the continents have grown during the past 2,500 Myr by anywhere from ~ 10 to 40%. Crustal growth is required as long as freeboard at the end of the Archaeon was not more than 400 m smaller than it is today. Crustal shrinkage since the end of the Archaeon requires an increase in freeboard by at least ~ 0.5 km since 2,500 Myr ago. Although secular freeboard changes of several hundred metres since the end of the Archaeon might be consistent with the geological evidence, changes of 0.5 km or more probably can be ruled out. Therefore, we conclude on the basis of Fig. 3 that post-Archaeon crustal growth by a few tens of per cent is required.

Cooling of the Earth and deepening of the ocean basins tend to increase freeboard through geological time. Crustal shrinkage (with constant thickness) reinforces this trend. Figure 3 shows that shrinkage by even as much as 10% since 2,500 Myr ago would mean that the continents just emerged above sea level at the end of the Archaeon. Crustal growth offsets the tendency of deepening ocean basins to increase freeboard with time. If the crust has grown more than 25% since the end of the

Table 1 Values of model parameters

Parameter	Definition	Value
V_0	Volume of oceans	$1.17 \times 10^{18} \text{ m}^3$
V_c^*	Present volume of continents	$7.6 \times 10^{18} \text{ m}^3$
A_c^*	Present area of continents	$2.17 \times 10^{14} \text{ m}^2$
A_0^*	Present area of oceans	$293 \times 10^{14} \text{ m}^2$
A_s	Surface area of Earth	$5.1 \times 10^{14} \text{ m}^2$
d_s^*	Present depth of mid-ocean ridge system below sea level	2,500 m
d_b^*	Present depth of ocean basins below the rise crests	1,500 m
d_c^*	Present average crustal thickness	38 km
h^*	Present continental freeboard	750 m
ρ_m	Mantle density	$3,300 \text{ kg m}^{-3}$
ρ_w	Water density	$1,000 \text{ kg m}^{-3}$
ρ_{cc}	Crustal density	$2,750 \text{ kg m}^{-3}$

Archaeon, freeboard has decreased during the Proterozoic-Phanerozoic, that is, the continents would have had their maximum emergence at 2,500 Myr ago. This runs against most assessments of the geological and sedimentological records, which support a constancy^{1,2,4-6} or a slight increase¹² rather than a decrease¹³ in continental freeboard since $\sim 2,500$ Myr ago.

To assess how variations in crustal thickness might affect our conclusions, we consider how freeboard and continental volume are related for prescribed $d_c(t)$

$$h - h^* = \frac{(\rho_m - \rho_w)}{(\rho_m)} \left[\frac{(\rho_m - \rho_{cc})}{(\rho_m - \rho_w)} (d_c - d_c^*) + d_s^* + d_b^* \frac{q_s^*}{q_s} - \frac{V_0}{(A_c - V_0/d_c^*)} \right] \quad (6)$$

With $d_c(t)$ from Fig. 2a, we use equation (6) to determine freeboard (or continental volume) given an assumed variation in V_c (or h). It can be deduced from equation (6) that crustal thickening augments the deepening of the ocean basins with time by requiring even larger increases in crustal volume (compared with d_c constant) to preserve constant freeboard.

Figure 2b illustrates how crustal thickening by 10 and 20% since 4,500 Myr ago modifies the amount of crustal growth required to maintain constant freeboard. Even a small amount of crustal thickening greatly increases the quantity of crustal growth necessary for constancy of freeboard. For example, with 10% crustal thickening over all of geological time, continental volume would have to have doubled since the end of the Archaeon. The geological evidence argues against such a large post-Archaeon increase in V_c .

Figure 2c summarizes how the assumed crustal thickening influences freeboard for no crustal growth. With V_c constant, a thinner crust in the past implies a larger continental area and, therefore, a smaller freeboard than at present. With even small increases in d_c , the continents would not rise above the oceans until at least the mid-Proterozoic. An increase in d_c and a late emergence of the continents have been proposed¹⁴⁻¹⁶, but this is not supported by geological data². A drastic global increase in d_c by underplating¹⁶ neglects the freeboard constraint and hotspot frequency data⁷, but underplating could be applicable to rapidly formed crustal segments¹⁷. We conclude that post-Archaeon changes in mean crustal thickness are highly unlikely.

Some secular changes in h may have occurred which are not included in our model, but their analyses are not useful as long as no firmer constraints can be put on the 'error' in constant freeboard. A regression during the Phanerozoic has been proposed¹², but the amount depends on an arbitrary point in time from which detailed data are available and does not necessarily point to a regression since the Archaeon¹⁸. A 5% change in ocean volume corresponds to a 200-m change in sea level. Therefore, small secular changes in ocean volume cannot be excluded. Present estimates of water subducted in trenches and released at mid-ocean ridges vary widely¹⁹ and need revision. Changes with time in the thickness of ocean floor sediments, in

the depth to ridge crests, in oceanic crustal thickness and in the development of deep mantle roots under the continents may all influence freeboard. Lack of data limits the usefulness of incorporating these effects into our model and increases the overall uncertainty in our results.

The continents increased their size during the Proterozoic and Phanerozoic^{7,17}, indicating at least 20% growth since 2,500 Myr ago. Such a growth rate is close to the requirement for constant freeboard and encourages us to believe that ocean volume and crustal thickness have remained essentially constant since 2,500 Myr ago, with error margins as given above.

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Sea-bed noises reveal role of turbulent bursting phenomenon in sediment transport by tidal currents

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In many engineering and sedimentological applications, it is usual to relate sediment movement to the time-averaged bed shear stress. However, the shear stress exerted on the sea bed by tidal currents is intermittent in nature due to the turbulent bursting phenomenon^{1,2}. Sediment movement at the sea bed also occurs in bursts^{3,4}. However, attempts to link these two processes have failed because of the poor temporal resolution of existing sediment-transport measurement techniques. Recent experimental advances now make this possible and we describe here the first detailed observations of sediment movement and fluid turbulence in tidal currents flowing over sandy gravels. These results show that high stress values are a necessary, but not sufficient, condition for bed-load sediment movement and that form drag on individual particles may have greater dynamical significance than the stress. This work also suggests that it may now be possible to describe sediment movement at a level which is deterministic in terms of the turbulence and at very much shorter timescales than have been used previously.

The importance of the bursting phenomenon in tidal current boundary layers has been confirmed in previous work^{1,2}. This has shown that, similarly to laboratory boundary layers^{5–8}, the bulk of the Reynolds stress is made up by contributions from large and intermittent momentum transporting events which, collectively with other weaker motions, comprise the bursting phenomenon. Here the Reynolds stress is defined as $\tau = -\rho u w$ where ρ is the fluid density, u and w are the horizontal and

vertical turbulent velocity fluctuations and the overbar denotes averaging in time. In particular, these early studies established that positive contributions to the stress were principally from two types of motion; ejections in which $u < 0$; $w > 0$ and sweeps in which $u > 0$; $w < 0$. In addition, two weaker motions, inward ($u < 0$; $w < 0$) and outward ($u > 0$; $w > 0$) interactions, made negative contributions to the stress. Note that the motions which contribute to the bursting sequence of events are generally considered to be associated with pairs of counter rotating vortices⁹. These, in turn, may be associated with a Taylor–Görtler instability¹⁰. However, the exact generation mechanism for the vortices and the bursting phenomenon is unknown⁹.

In parallel with the purely fluid dynamical interest, there has been considerable speculation^{11–14} over the exact role of the turbulent bursting phenomenon in sediment transport. However, only recently has the development of a passive acoustic technique made it possible to observe sediment movement with a temporal resolution comparable with the high frequency turbulence in the flow. In particular, it is possible to detect^{15–17} the self-generated noise (SGN) arising from interparticle collisions of mobile sediment and to relate the acoustic intensity to the amount and size of sediment which is moving. Thus, for the first time, it has been possible to make a detailed examination of the relationship between bed-load transport and the turbulent structure of the boundary layer near the sea bed.

During studies to examine the incipient motion of sea-bed gravels in the West Solent, on the south coast of England⁴, measurements have been made of the horizontal (u) and vertical (w) turbulent velocity fluctuations at a height of 0.33 m above the sea bed using electromagnetic current meters¹⁵. The water depth in the study area was ~14 m. Underwater television (TV) and a wideband (–3 dB ≈ 150 kHz) hydrophone were used to monitor the movement of sediments in the approximate size range 4–32 mm (ref. 4). The hydrophone was positioned at a height of 0.24 m above the sea bed. Turbulent flow and SGN data were recorded at 0.2-s intervals. (Further details of these measurements are given in refs 4, 15.)

Figure 1 shows measurements of the horizontal and vertical turbulent velocity fluctuations u and w , and their contribution $-uw$ to the Reynolds stress. Figure 1 confirms the presence of the bursting phenomenon in tidal currents flowing over gravels in the West Solent with instantaneous stress values which may be 10 or more times greater than the mean value obtained by averaging the stress over a typical record length of 1,200 s (Table 1). Figure 1 shows that the bursting phenomenon is uniquely identified by the large and intermittent contributions that it makes to the Reynolds stress. This intermittency occurs despite the fact that u and w are normally distributed and randomly fluctuating quantities when taken separately¹⁸. Figure 1 also shows the SGN signal, corrected for background noise, and illustrates that the bulk of the gravel movement occurs in bursts, during sweep type events. This is illustrated further in Fig. 2, which shows u , w , $-uw$ and the SGN signal on an expanded timescale. Figure 2a shows ejection type events ($u < 0$; $w > 0$) which produce no sediment movement while Fig. 2b shows sweep events ($u > 0$; $w < 0$) producing appreciable quantities of sediment movement. Underwater TV observations¹⁵ of gravel movement have provided an independent means of checking the performance of the SGN device as shown in Fig. 1. As far as we know this is the first description of observations of the type shown in Figs 1 and 2.

To examine in more detail the relationship between sediment movement and turbulent fluctuations in the Reynolds stress, comparisons have been made of the amounts of sediment movement occurring in the different types of Reynolds stress event. For these comparisons five records from the West Solent were chosen, corresponding to a period of decelerating tidal flow on 19 September 1982 (Table 1).

Acoustic intensity levels were compared with the stress ($-uw$) records, following linear trend removal from u and w , an empirically determined 1-s adjustment for lag effects due to the separation between the flow sensors and the sea bed, and possible

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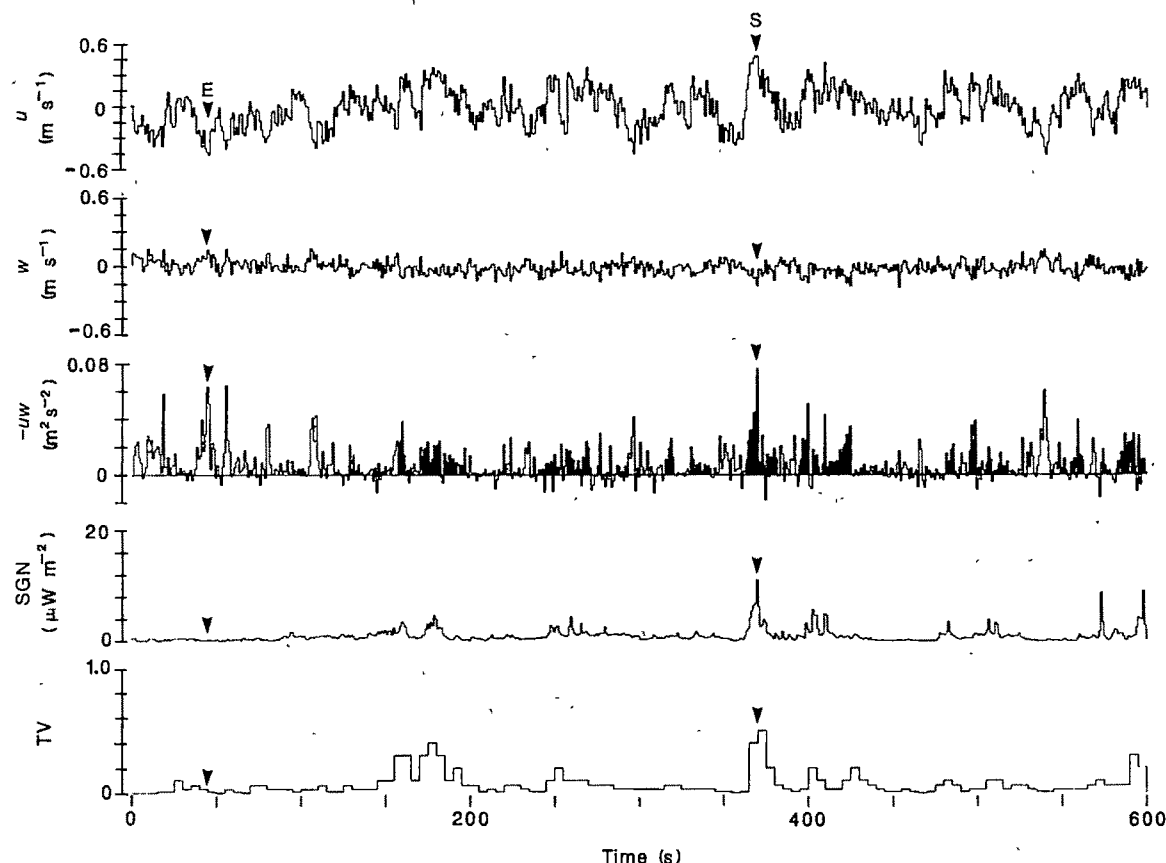


Fig. 1 Records of the horizontal and vertical turbulent velocity fluctuations u and w , and their product $-uw$. Also shown is the SGN acoustic intensity record and qualitative estimates of gravel movement from the underwater TV. For clarity 1-s averages only of u , w and SGN are shown together with 5-s estimates of gravel movement from the underwater TV. Black stress events (S) correspond to sweeps ($u > 0$; $w < 0$) and white events (E) correspond to ejections ($u < 0$; $w > 0$).

particle inertia. The significant stress events in each record were identified using a procedure similar to that given in refs 19, 20. Stress events were ranked according to peak size and regardless of sign, in such a way as to make up a total contribution of 90% to the overall stress. Event duration was defined by forwards and backwards interpolation to give uw values which were 10% of each peak. Event amplitude was defined as the average of $-uw$ taken over the duration of the event. Average values of u , w and the acoustic intensity were also calculated over the same time interval. Stress events were then identified as ejections, sweeps and inward or outward interactions according to the sign of the u and w averages. In this way, the average acoustic intensity levels, and hence the quantity of sediment moved by the significant stress events in each record, could be categorized and compared. Further details of the amplitudes, durations and percentage contributions to the overall stress, for the various events, are given in Table 1.

The results of this analysis are shown in Fig. 3, which shows

comparisons for 3,187 events measured over a period of ~ 100 min. This demonstrates quite clearly (Fig. 3a, b) that acoustic intensity levels associated with sweeps ($u > 0$; $w < 0$) are substantially higher than the intensity levels during ejections ($u < 0$; $w > 0$) at high stress values. This difference increases as the stress ($|uw|$) increases. From this we conclude that of the two types of motion which contribute to the bulk of the Reynolds stress, ejections and sweeps (Table 1), only sweeps are capable of supporting appreciable sediment movement. However, an interesting and unexpected result is that outward interaction events ($u > 0$; $w > 0$), although weaker and less frequent than sweeps (Fig. 3e), are capable of supporting greater sediment movement in the stress range $0 < |uw| < 0.04 \text{ m}^2 \text{ s}^{-2}$. This is despite the fact that they make a negative contribution to the stress. Correspondingly, there is little sediment movement associated with inward interactions ($u < 0$; $w < 0$). On average, sweeps and outward interactions contribute $\sim 46\%$ of the overall stress and 73% of the total acoustic intensity in only 24% of the time.

Table 1 Details of records used in comparisons of SGN acoustic intensity levels and contributions to the Reynolds stress (Fig. 3)

Record (h)	U_{100} (m s^{-1})	$-\overline{uw}$ ($\text{m}^2 \text{ s}^{-2}$)	No. of events				Duration of events (s)				Overall mean size (stress) ($\text{m}^2 \text{ s}^{-2}$)				Overall mean size (SGN) ($\mu\text{W m}^{-2}$)				Contribution to stress (%)			
			E	S	OI	II	E	S	OI	II	E	S	OI	II	E	S	OI	II	E	S	OI	II
0924-	1.23	0.0087	249	236	57	54	0.91	0.91	0.61	0.66	0.0201	0.0229	-0.0129	-0.0101	0.6345	2.2694	2.9384	0.5437	47.40	50.19	-4.21	-3.34
0944							(0.58)	(0.53)	(0.65)	(0.31)	(0.0104)	(0.0147)	(0.0058)	(0.0058)	(0.9329)	(3.1589)	(3.6808)	(0.6895)	(20.99)	(19.95)	(3.23)	(3.30)
0944-	1.27	0.0077	218	223	41	40	0.86	0.85	0.56	0.64	0.0188	0.0197	-0.0109	-0.0107	0.7936	1.4854	1.6443	0.6148	48.93	47.71	-2.96	-3.56
1001							(0.52)	(0.45)	(0.22)	(0.26)	(0.0109)	(0.0114)	(0.0045)	(0.0051)	(1.0129)	(1.4313)	(1.3794)	(0.7229)	(19.48)	(19.38)	(2.33)	(2.63)
1024-	1.16	0.0081	239	239	62	67	0.92	0.87	0.61	0.71	0.0207	0.0220	-0.0135	-0.0130	0.4452	1.3669	1.6727	0.5072	51.12	50.13	-5.22	-6.01
1044							(0.56)	(0.50)	(0.30)	(0.36)	(0.0104)	(0.0120)	(0.0047)	(0.0063)	(0.6413)	(1.8723)	(1.6221)	(1.2019)	(19.31)	(18.48)	(3.30)	(4.12)
1044-	1.11	0.0064	284	295	119	126	0.85	0.82	0.59	0.71	0.0145	0.0162	-0.0099	-0.0086	0.2776	0.6353	0.6661	0.2262	51.54	57.20	-8.69	-10.02
1104							(0.54)	(0.50)	(0.30)	(0.39)	(0.0101)	(0.0102)	(0.0070)	(0.0049)	(0.3392)	(0.8660)	(0.7863)	(0.3545)	(21.02)	(20.94)	(6.07)	(7.74)
1104-	1.08	0.0070	222	259	78	64	1.04	0.88	0.53	0.58	0.0163	0.0174	-0.0110	-0.0114	0.1129	0.4199	0.4098	0.1017	51.02	49.27	-5.34	-4.92
1124							(0.75)	(0.50)	(0.19)	(0.23)	(0.0082)	(0.0092)	(0.0060)	(0.0061)	(0.2220)	(0.6159)	(0.6224)	(0.1311)	(21.06)	(20.57)	(3.76)	(3.36)

U_{100} is the current at 1 m above the sea bed from near-bed velocity profile measurements. The water depth at this location was ~ 14 m. $-\overline{uw}$ is the overall kinematic stress. The Reynolds stress is given by $\tau = -\rho \overline{uw}$. All the records were obtained in the West Solent on 19 September 1982. Record lengths are 20 min with the exception of the record beginning 0944 h (AST) which is 17 min in length. E denotes ejections, S sweeps, OI outward interactions and II inward interactions. Values in parentheses are standard deviations except in the case of contributions to the stress where they indicate the proportions of time as a percentage.

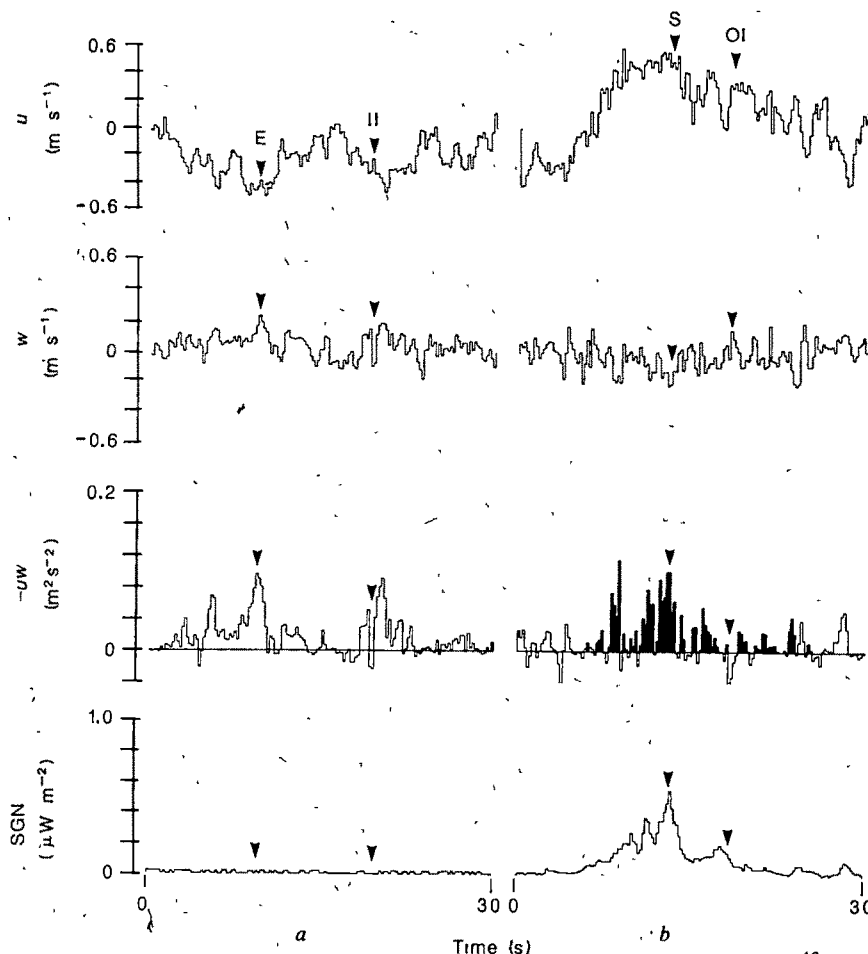
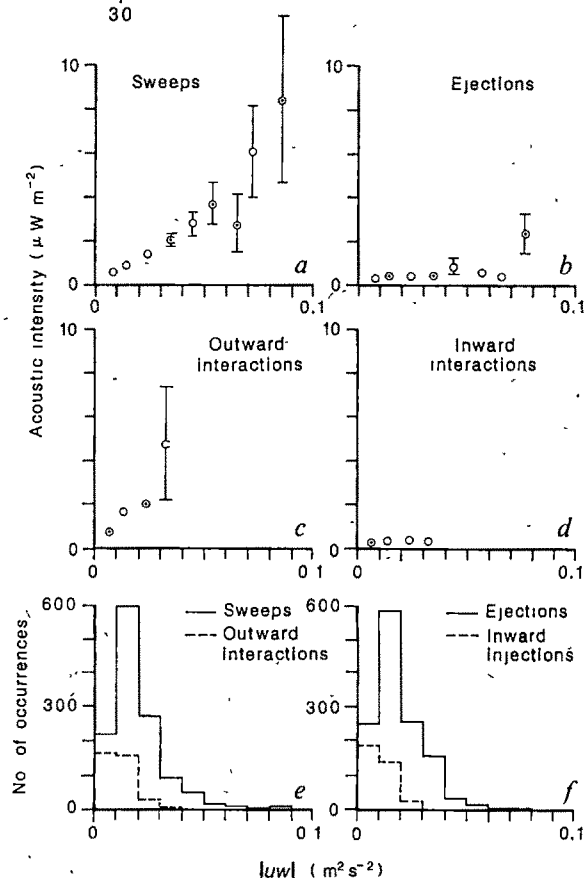


Fig. 2 (Left) u , w , $-uw$ and SGN records on an expanded timescale showing: *a*, ejection-type events (E) which produce no sediment movement; and *b*, similar-size sweep (S) events (black) producing appreciable sediment movement. Inward and outward interaction events, II and OI respectively, are also indicated. Only 5-Hz data are shown.

Fig. 3 (Right) Comparisons of sediment movement due to sweeps (*a*), ejections (*b*) and outward and inward interactions (*c* and *d*). *a-d* show mean values of the acoustic intensity in each $0.01 \text{ m}^2 \text{ s}^{-2}$ interval of $|uw|$, plotted against the mean stress in the same range. The number of events comprising each mean value of the acoustic intensity is indicated in *e* and *f*. Observations with <2 events are not shown. Error bars correspond to ± 1 s.e.m. and are not shown for standard errors $<0.2 \text{ } \mu\text{W m}^{-2}$. Standard error bars for the stress were $<4\%$ of each mean and for clarity are not shown. The total number of events was 3,187.

These results suggest that horizontal turbulent velocity fluctuations (u) may have greater dynamical significance in terms of sediment movement, than the instantaneous contributions ($-uw$) to the Reynolds stress. However, vertical fluctuations (w) may still be important provided they are associated with increases in u . This seems to be the case for outward interactions (Fig. 3c), where $w > 0$ may indicate additional lift on exposed gravel particles by fluid moving away from the bed. This, in turn, may account for sediment transport rates which are higher than those in similar-size sweeps.

The observed close dependence on u could be explained if the gravel was moved principally by form drag acting on the flow-normal projected area of exposed particles. Sediment sampling²¹ has confirmed that the West Solent gravels are poorly sorted and underwater TV observations⁴ have shown that sediment movement is by sliding or rolling of exposed particles. To test this hypothesis, we have carried out correlation analyses on the acoustic intensity and turbulent flow records in Table 1, first in terms of the form drag proportional to $(\bar{U} + u)^2$, where $\bar{U}$ is the horizontal mean flow, and second in terms of contributions $|uw|$ to the Reynolds stress ($-puw$). The results of these comparisons are shown in Table 2 and indicate that in all cases sediment movement is better correlated with form drag than with the instantaneous stress. Note that although the correlation coefficients in Table 2 are low, they are, nevertheless, highly significant because of the very large number of data points in



each record (about 6,000). On average, Table 2 shows that the correlation (r) of sediment movement with $(\bar{U} + u)^2$ is a factor of two times better than that with $|uw|$.

Through the application of a novel acoustic technique, this work has provided a detailed high temporal resolution comparison of sediment response and turbulent flow conditions.

Table 2 Results of correlation analyses of SGN records with $(\bar{U} + u)^2$ and $|uw|$

Record (h)	Correlation coefficient (r) at 1 s lag SGN/ $(\bar{U} + u)^2$	SGN/ $ uw $
0924-0944	0.45	0.26
0944-1001	0.30	0.15
1024-1044	0.39	0.19
1044-1104	0.35	0.17
1104-1124	0.33	0.12

This has shown that bed-load movement of sea-bed gravels is caused principally by sweep-type motions in the bottom boundary layer and to a lesser extent by outward interactions. This observation can be explained if form drag rather than shear stress is assumed to be the principle cause of gravel movement. Furthermore, laboratory observations^{22,23} in steady currents have shown that the movement of finer sediments in suspension may also be linked to the turbulent bursting process. It is, therefore, interesting to speculate that a complete description of sediment movement in terms of the fluid turbulence might now be possible. In conceptual terms at least, this would consist of bed-load transport of coarse sediments driven principally by sweep-type events, while suspended load transport of finer sediments would be dominated by the ejection of low-momentum fluid away from the boundary. Beyond this, if sediment movement can be quantified in terms of the magnitude of sweep- and

ejection-type events, and if the frequency with which these motions occur can be predicted accurately⁹, then a new generation of sediment transport equations may be deduced which take the turbulent bursting process into account.

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Hydrothermal particle plumes over the southern Juan de Fuca Ridge

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Hydrothermal particles originate in buoyant plumes emanating from seafloor thermal vents¹⁻⁴ and accumulate as metalliferous sediments found along oceanic spreading centres⁵⁻⁷. Observational evidence of the transport pathway of hydrothermal particles is scarce, however, and the structure, extent and particle concentrations of hydrothermal plumes remain largely conjectural. To evaluate the potential of hydrothermal plumes as particle transport agents, we mapped their distribution along and across the 80-100-m-deep axial valley of the southern Juan de Fuca Ridge in the north-east Pacific Ocean ($\sim 44^{\circ}30'N$, $130^{\circ}W$), an area of known hydrothermal activity⁸⁻¹¹. Continuous light-scattering measurements, reported here, defined numerous particle plumes centred 30-120 m above bottom (m.a.b.); elemental analysis of the plume particles and the θ (potential temperature)- S (salinity) signatures of the plume water verified their hydrothermal origin. Concentrations of hydrothermal particles remained elevated at ridge-crest depths at least 100 km from the ridge, indicating distant off-axis dispersal of hydrothermal emissions.

Two-dimensional plume representations were constructed from continuous data acquired by an acoustically-tracked sensor package regularly cycled between 20 and 200 m.a.b. while being towed. Twenty-six tows along or across an 18-km segment of the axial valley were made between 27 May and 17 June 1984 (Fig. 1). Sensors on the tow body included an integrating scatterance meter (nephelometer), thermometer (resolution $\pm 0.0003^{\circ}C$), and conductivity cell (resolution $\pm 0.00004 S m^{-1}$). In addition to the tows, vertical conductivity-temperature-depth (CTD)/nephelometer/rosette casts over the ridge axis and up to 100 km off axis further defined plume distributions and collected particles for chemical analysis by secondary emission X-ray fluorescence¹² and for empirical calibration of the nephelometer. Relative light scattering values were linearly and significantly correlated (correlation coefficient, $r^2 = 0.86$) with mass con-

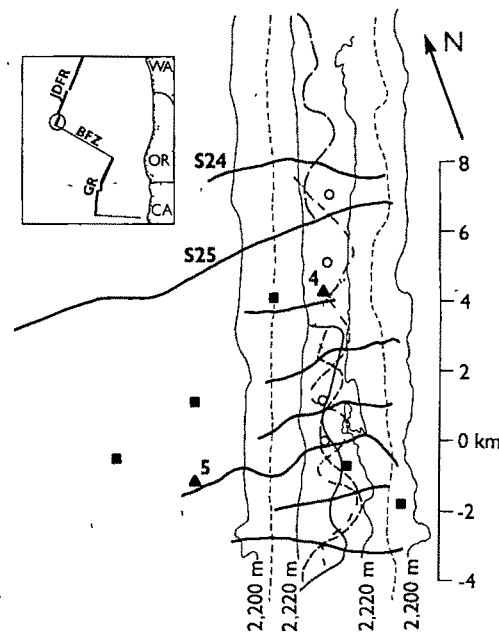


Fig. 1 The study area, circled in the inset, lies along the Gorda Ridge-Blanco Fracture Zone-Juan de Fuca Ridge rift system of the north-east Pacific Ocean. Selected tow tracks are shown relative to the floor of the flat axial valley (delimited by the 2,220-m contours) and the adjacent ridge crests (indicated by the thin dashed lines). The axial strike is $\sim 18^{\circ}$. Individual along-axis tow tracks can be identified by the key in Fig. 2b; cross-axis tracks are shown as thick solid lines. The locations of known active hydrothermal vents ($\circ$) as determined by deep-tow⁹ and submersible¹⁰ observations are shown along the centreline of the valley. Positions of moorings ($\blacktriangle$) and selected CTD casts ($\blacksquare$) are also shown. Sediment traps and current meters were located 50 m.a.b. on V4 and 100 m.a.b. on V5, an additional current meter was at 200 m.a.b. on V4.

centration determined by membrane filtration of 129 samples. Vertical particle flux (from sediment traps¹³) and current speeds were measured during the cruise on moorings in the axial valley (V4) and 4 km west of the valley (V5).

Each of the 26 tows found particulate plumes derived from hydrothermal emissions. The hydrothermal origin was indicated

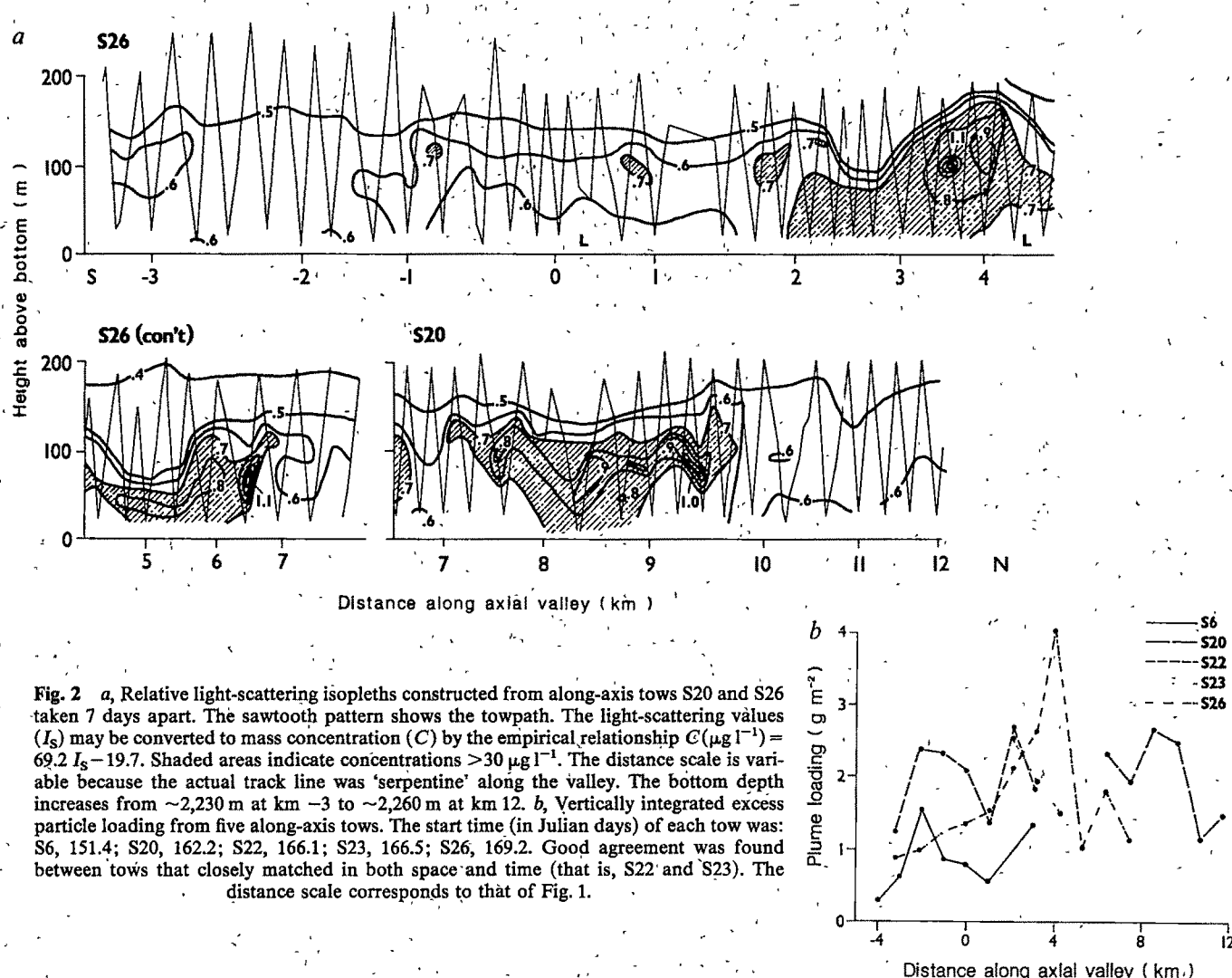


Fig. 2 a, Relative light-scattering isopleths constructed from along-axis tows S20 and S26 taken 7 days apart. The sawtooth pattern shows the towpath. The light-scattering values (I_s) may be converted to mass concentration (C) by the empirical relationship $C(\mu\text{g l}^{-1}) = 69.2 I_s - 19.7$. Shaded areas indicate concentrations $>30 \mu\text{g l}^{-1}$. The distance scale is variable because the actual track line was 'serpentine' along the valley. The bottom depth increases from $\sim 2,230$ m at km -3 to $\sim 2,260$ m at km 12. b, Vertically integrated excess particle loading from five along-axis tows. The start time (in Julian days) of each tow was: S6, 151.4; S20, 162.2; S22, 166.1; S23, 166.5; S26, 169.2. Good agreement was found between tows that closely matched in both space and time (that is, S22 and S23). The distance scale corresponds to that of Fig. 1.

by departures of up to 0.1°C from the local θ -S curve and (on casts) by particulate Fe/Al ratios enriched up to 79 times the background value of ~ 1 . Light-scattering observations, however, provided greater sensitivity than temperature in identifying hydrothermal plumes and far greater resolution than discrete chemical samples in describing them. Particle concentrations within the axial valley and off-axis plumes were elevated 2–10 times above the background particle concentration of $\sim 8 \mu\text{g l}^{-1}$ determined from mid-depth water samples. The horizon of maximum particle concentration in the axial valley characteristically occurred 30–120 m.a.b. (Fig. 2a) and was punctuated by localized maxima exceeding $35 \mu\text{g l}^{-1}$ and vertical gradients up to $5 \mu\text{g l}^{-1} \text{ m}^{-1}$. Well-defined plumelike features such as that centred at km 8.5 of tow S20 (Fig. 2a) were rare and restricted to a horizontal dimension of 1–3 km. Intense concentration maxima ($>40 \mu\text{g l}^{-1}$) preferentially occurred in the northern half of the study area and were separated by intervals of 2–3 km; this distribution conforms to the distribution of active vent fields documented by deep-tow photography⁹ and submersible observations¹⁰.

The concentration of particles originating solely from plume emissions was estimated by subtracting the background value of $8 \mu\text{g l}^{-1}$ from all transects and vertically integrating the resulting excess mass loading. These calculations will exceed the true emission loadings by the amount that resuspension of non-hydrothermal sediment contributes to the observed concentrations, but maximum current speeds of only 14 cm s^{-1} (V4) and the prevalence of above-bottom particle maxima suggest that resuspension was weak. Plume loadings calculated at 1-km intervals along five tows show marked spatial and temporal variability (Fig. 2b), consistent with the pattern expected from

discrete vent fields whose emissions are advected by a variable current regime. Temporal changes in discharge rates could also influence the interpretation of the observations.

After documenting the presence of hydrothermal plumes within the axial valley, we conducted eight cross-axis tows to evaluate the likelihood of off-axis dispersion of the particles. The tows were spaced at ~ 2 -km intervals along the valley (Fig. 1); all but the most southerly two found asymmetric plumes of varying intensity apparently originating along the centre or west side of the valley and extending up to, and sometimes over, the west ridge crest. (A particle plume at ridge-crest depths was also detected up to 10 km east of the valley by individual CTD casts.) Particle concentration isopleths along all of these tows roughly paralleled the bathymetry. The longest tow, S25, traced a continuous plume for >8 km west of the axial valley (Fig. 3a). The hydrothermal origin of this feature was confirmed by the continuity of elevated Fe/Al ratios of collected particles westward from the valley (Fig. 3a). Significant maxima at ridge-crest depths in both particle concentration and Fe/Al ratios were also observed on CTDs 20 km and 100 km to the west, suggesting that crestal emissions spread away from the ridge along the isopycnal $\sigma_\theta = 27.72$.

In contrast to these distinct off-axis trends, mean velocities at V4 and V5 during the cruise were weak ($1\text{--}2 \text{ cm s}^{-1}$) and directed northwards roughly parallel to the ridge axis. Mean cross-axis currents were $<5\%$ of along-axis currents within the valley (50 m.a.b. at V4) but increased to 25% 200 m.a.b. at V4 and to 20% at V5. Tidal currents (V4) were dominated by the M2 component, which had a major-axis amplitude of $\sim 4 \text{ cm s}^{-1}$, oriented along the ridge, with an ellipticity of ~ 0.1 .

The presence of a plume several kilometres west of the valley

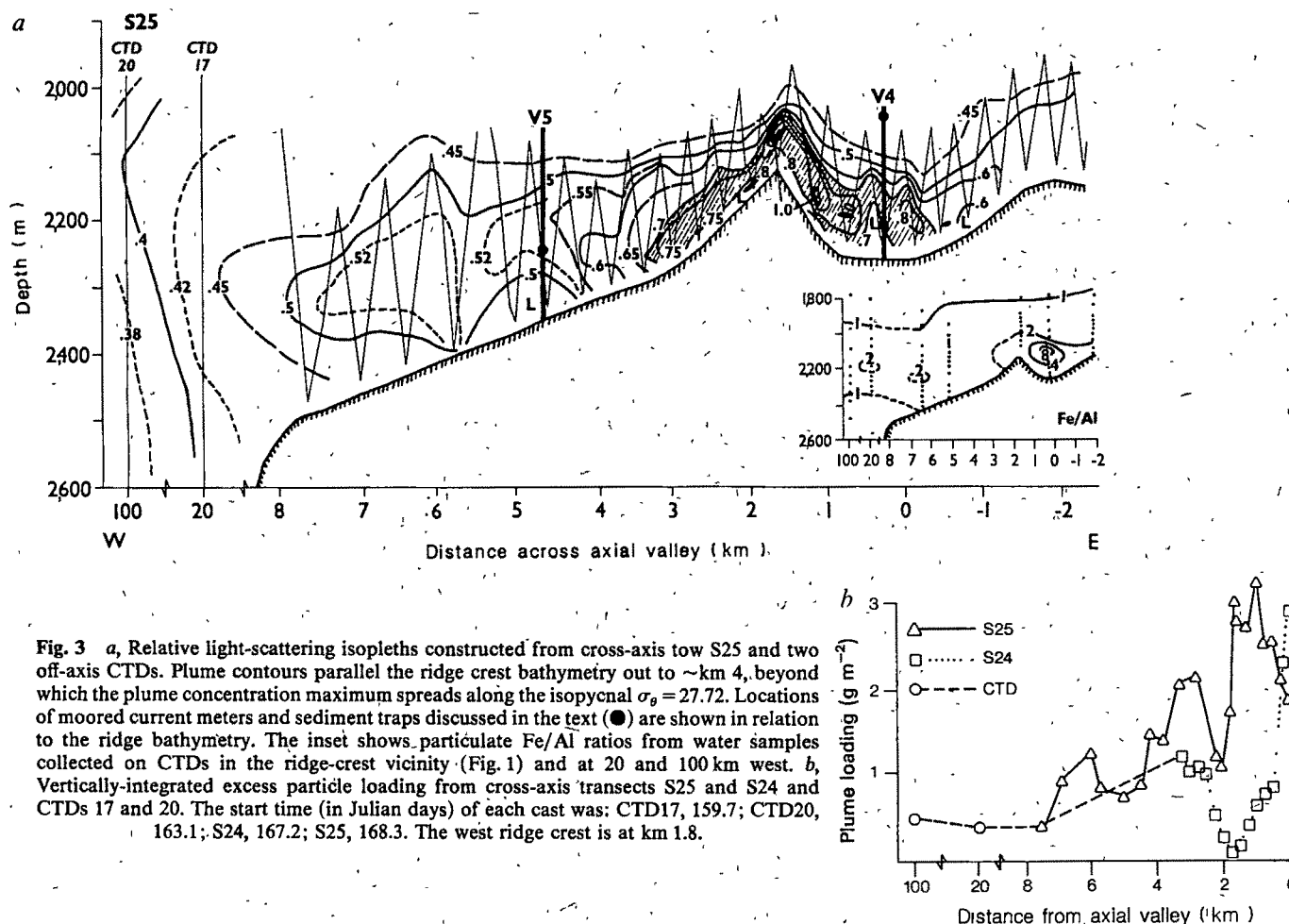


Fig. 3 *a*, Relative light-scattering isopleths constructed from cross-axis tow S25 and two off-axis CTDs. Plume contours parallel the ridge crest bathymetry out to ~km 4, beyond which the plume concentration maximum spreads along the isopycnal $\sigma_\theta = 27.72$. Locations of moored current meters and sediment traps discussed in the text (●) are shown in relation to the ridge bathymetry. The inset shows particulate Fe/Al ratios from water samples collected on CTDs in the ridge-crest vicinity (Fig. 1) and at 20 and 100 km west. *b*, Vertically-integrated excess particle loading from cross-axis transects S25 and S24 and CTDs 17 and 20. The start time (in Julian days) of each cast was: CTD17, 159.7; CTD20, 163.1; S24, 167.2; S25, 168.3. The west ridge crest is at km 1.8.

in the face of predominantly northward advection suggests that the plume had multiple sources. We speculate that particles from vent fields close to the S25 track caused the within-valley plume, while particles from more southerly vent fields crossed the track several kilometres west (and east) of the ridge crest. The vertically-integrated particle loading along S25 (Fig. 3b) shows a major peak on the western edge of the valley, and two lesser peaks west of the ridge crest that could be indicative of separate, southerly sources. Tow S24, taken 1 day earlier just to the north of S25, shows a similar loading increase, west of the ridge crest, that could have been continuous with the S25 plume (Fig. 3b). The paucity of our coverage, however, means that these results might have other interpretations.

If particle production by the vent fields was approximately steady state during the cruise, and if our off-axis transects were representative of the entire ridge, the off-axis loading decrease must result primarily from settling and/or horizontal dispersion. The contribution of settling can be evaluated from the vertical flux of hydrothermal material. The total particle flux into the sediment traps was $0.11 \pm 0.04 \text{ g m}^{-2} \text{ day}^{-1}$ for 10 two-day samples at V4, and $0.19 \text{ g m}^{-2} \text{ day}^{-1}$ for a single 10-day sample at V5. Elemental analysis indicates that the trapped material was predominantly biogenic and aluminosilicate, with <10% of the total attributable to hydrothermal phases. If a flux of $\leq 0.01 \text{ g m}^{-2} \text{ day}^{-1}$ is thus characteristic of hydrothermal phases in the study area, settling can have only a minor impact on the observed plume loadings. We note, however, that this flux could support the hydrothermal phase accumulation rates of $0.001\text{--}0.004 \text{ g m}^{-2} \text{ day}^{-1}$ found in the vicinity of the Juan de Fuca Ridge¹⁴.

We infer from these observations the following model of hydrothermal plume distributions around the southern Juan de Fuca Ridge. Buoyant vent emissions create a particle distribution that is vertically stratified around above-bottom concentration maxima. Along-axis variability arises because vent fields are not

continuous along the axial valley. Cross-axis tows and CTDs demonstrate that hydrothermal particles are effectively transported off axis, even though the net advective direction is northward, within roughly $\pm 20^\circ$ of the axial strike. We hypothesize that the loss rate of suspended hydrothermal phases by settling is slow enough to enable diffusion to coalesce individual plumes into a laterally uniform far-field hydrothermal cloud at distances of 20–100 km from the ridge crest, analogous to the cloud formed by dissolved tracers such as ^3He (ref. 15) and Mn (ref. 16).

This study has provided an initial description of the distribution of suspended hydrothermal particles; determination of a regional mass-balance between suspended and deposited particles will require quantification of both the percentage of emissions that escapes local deposition and becomes suspended in a plume, and the rate of *in situ* particle-production by slow precipitation of dissolved species within the advecting plume.

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Acetylcholine activates an inward current in single mammalian smooth muscle cells

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Acetylcholine, the major excitatory neurotransmitter to the smooth muscle of mammalian intestine¹, is known to depolarize smooth muscle cells with an apparent increase in membrane conductance². However, the ionic mechanisms that are triggered by muscarinic receptor activation and underlie this response are poorly understood, due in part to the technical problems associated with the electrophysiological study of smooth muscle³. The muscarinic action of acetylcholine in certain neurones has been shown to involve the switching off of a resting K^+ current (M-current)⁴ and a similar mechanism has recently also been identified in smooth muscle of amphibian stomach⁵. We have now applied the patch-clamp technique⁶ to single smooth muscle cells^{7,8} of rabbit jejunum and find that muscarinic receptor activation switches on a non-selective, voltage-sensitive inward current. In addition, acetylcholine activates and then suppresses spontaneous K^+ current transients, which are probably triggered by rises in intracellular Ca^{2+} in these cells.

Membrane current and voltage recordings were made from enzymatically dispersed single cells isolated from the longitudinal smooth muscle layer of adult rabbit jejunum⁷. These cells were 5–15 μm wide, up to 300 μm long and contracted on application of acetylcholine. Acetylcholine was applied either in the bathing solution or by iontophoresis from a glass micropipette placed about 10 μm from the mid-region of partially contracted cells, chosen to ensure uniform potential during voltage clamp (Fig. 1). Membrane currents and membrane potentials were recorded with a glass pipette in the 'whole-cell' recording configuration⁶.

In normal physiological saline solution, acetylcholine applied iontophoretically or in the bathing solution (0.1–10 μM) evoked contraction, membrane depolarization associated with an increase in membrane conductance (indicated by a reduction in size of hyperpolarizing electrotonic potentials) and action potential discharge (Fig. 1a). Thus, these single cells show similar membrane potential responses to those of cells within intact strips of smooth muscle tissue recorded with other techniques². During voltage-clamp, acetylcholine evoked an inward current at holding potentials between –40 and –80 mV (Figs 1b, 2a,b). This inward current cannot be explained as a decrease of a resting outward K^+ current, as input resistance decreases and there is an increase in current noise indicating that additional channels were being opened rather than already open ones being closed (Fig. 1a, c). To further investigate the mechanism of action of acetylcholine, we applied it to cells in solutions with elevated K^+ concentrations clamped at the K^+ equilibrium potential, E_K , so eliminating any possibility of a contribution from changes in K currents under these conditions. Figure 1c shows a response obtained from a cell bathed in 45 mM K^+ and voltage clamped at E_K (–25 mV). A large inward current was still generated by acetylcholine application, showing that inward current was activated which at this potential could not be due to K^+ ion flow.

Further analysis of the ionic selectivity of this current required the measurement of responses from the same cell at various membrane potentials, and this was made more difficult by the slowness of the response and its decline in size upon repetition. However, by bathing cells in elevated K^+ -containing solutions, we were able to obtain stable recordings for up to 40 min that allowed several responses such as those shown in Fig. 2a to be recorded in the same cell. At E_K (–25 mV) the inward current response was close to its maximum of about 30 pA after about

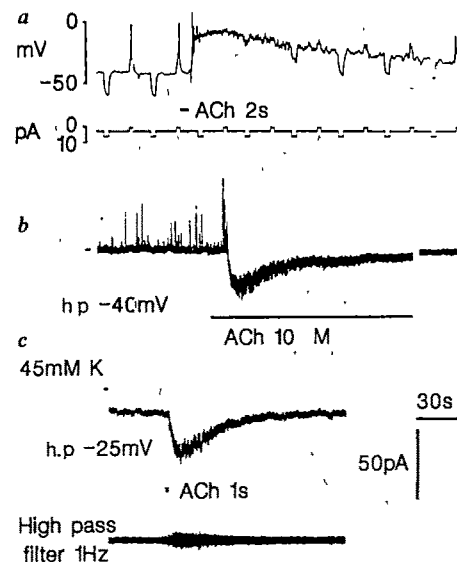


Fig. 1 Membrane potential and membrane current responses of jejunal smooth muscle cells to acetylcholine (ACh) application. **a**, Membrane potential record from a single cell with a small holding current applied to suppress spontaneous action potential discharge. Current pulses of alternating polarity were injected to evoke electrotonic potentials, and the depolarizing pulses elicited action potentials. ACh (2 s, 50 nA) was applied from an iontophoretic pipette positioned about 10 μm from the mid-region of the cell. This cell had an input resistance of 4.5 G Ω and cell capacitance of 70 pF (calculated from the hyperpolarizing electrotonic potential), which indicates a surface area of 6,700 μm^2 if 10^{-6} F cm^{-2} is assumed for specific membrane capacitance. This gives a space constant at the resting membrane resistance of about 5 mm, which is 60 times greater than the length of the short cable in these single cells. Thus, cells under voltage-clamp will be close to isopotential even with a substantial increase in conductance during acetylcholine application. The cell was bathed in a normal physiological saline solution containing (mM) Na^+ 125; K^+ 6; Ca^{2+} 2.5; Mg^{2+} 1.2; Cl^- 133; HPO_4^- 1.2; HEPES 10; glucose 11. The pipette (intracellular) solution contained (mM) Na^+ 5; K^+ 126; Mg^{2+} 1.2; Cl^- 128; HPO_4^- 1.2; EGTA 0.77; glucose 11; HEPES 10. All solutions were buffered to pH 7.2 and the temperature was 25 °C. **b**, Membrane current response of a cell under voltage-clamp to bath application of acetylcholine when the holding potential (h.p.) was –40 mV. Cell was bathed in same solution as **a**. **c**, Membrane current response of another cell bathed in 45 mM K^+ solution (K^+ replacing Na^+) at the calculated K^+ equilibrium potential (–25 mV). The lower trace shows the same response high-pass filtered at 1 Hz to show the increase in current noise on acetylcholine application. This increase in current noise is most simply explained by the opening and closing of channels activated by acetylcholine responsible for the inward current.

5 s and had a latency of around 500 ms. This current was reduced in amplitude at both more negative and more positive potentials. Figure 2b shows the current needed to clamp the cell at various potentials before and during the effects of acetylcholine application. The two extrapolated lines intersect just positive to 0 mV. This extrapolated E_{rev} does not correspond to the equilibrium potential of any one ion, indicating that the current is carried by a mixture of ions—probably K^+ , Na^+ and/or Ca^{2+} as has been concluded from work on whole smooth muscle tissue^{1,2}. The net agonist-induced current plotted against potential in this elevated K^+ concentration was U-shaped due to deviation from an ohmic conductance at potentials negative to –20 mV (Fig. 2c). Also shown are the averaged responses of 15 cells in normal (6 mM K^+) solution. The shape of the relationship appeared to be similar although shifted to the left, as might be expected from a leftward shift in E_K if K^+ was a permeant ion. Records obtained in this solution in the most interesting range of –30 to 0 mV, where the reversal potential probably lies, were complicated by the appearance of a burst of transient outward currents upon acetylcholine application (see below).

In solutions containing 45 mM K^+ , we had a better estimate

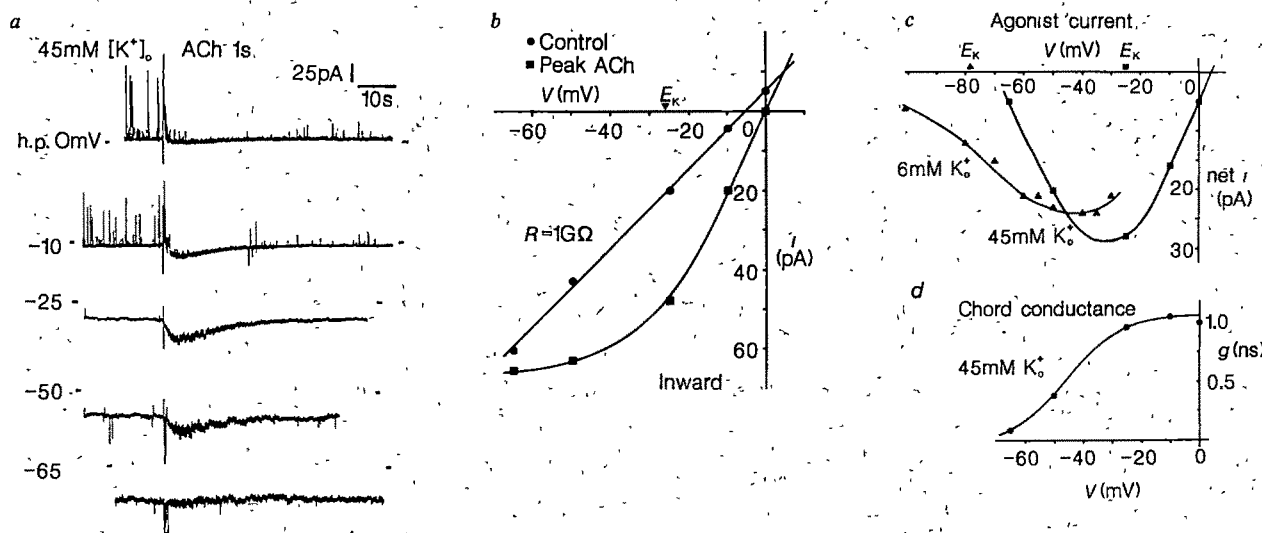


Fig. 2 *a*, Membrane current responses recorded from a single cell under voltage-clamp at five different holding potentials. Responses to 1-s iontophoretic pulses of acetylcholine were evoked at intervals of 8 min. Small horizontal bars indicate zero current level. Note the transient currents that appear as vertical deflections at holding potentials (h.p.) of 0, -10 mV (when they are outward) and -50, -65 mV (when they are inward); they are not seen at E_K (-25 mV). On acetylcholine application their frequency is temporarily increased and they may summate to produce an initial K^+ current that precedes the slower acetylcholine-induced current; after their initial potentiation the transient currents are inhibited for minutes. *b*, Current required to hold this cell at various potentials in the absence and presence of acetylcholine plotted from responses shown in *a*. The acetylcholine-induced current is maximal close to E_K and declines at potentials positive and negative to E_K ; it has an extrapolated reversal potential of about +5 mV. *c*, Net agonist-induced current at various potentials plotted from responses shown in *a* (■), and means of 33 responses from 15 cells in normal physiological saline (▲) $n = 2-7$. *d*, Chord conductance of responses shown in *a* using the extrapolated reversal potential under these conditions of +5 mV. The slope of the conductance curve between -50 and -25 mV gave an e-fold shift per -27 mV.

of E_{rev} at +5 mV (Fig. 2c) and this enabled the chord conductance of the current to be calculated. This showed that the acetylcholine-evoked current increased over the range -70 to -20 mV. The same type of voltage sensitivity, which could produce a regenerative depolarization, is produced by glutamate acting on *N*-methyl-D-aspartate (NMDA) receptors in certain neurones⁹. The non-ohmic properties of the NMDA current have recently been shown to be due to block of the channels at negative potentials by Mg^{2+} ions^{10,11}. We have not observed any effect of removing external Mg^{2+} on responses to acetylcholine, which suggests that the voltage dependence was not due to Mg^{2+} block. When instantaneous hyperpolarizing voltage jumps from -40 to -70 mV were applied at the peak of the acetylcholine response, an inward current was observed that decayed to a steady state with a $t_{1/2}$ of about 5 ms.

Acetylcholine application produced other changes in membrane currents. Spontaneous transient outward currents of varying amplitude, about 50 ms duration and with a frequency of 0.5-3 Hz were observed in cells bathed in elevated K^+ solution with normal calcium concentration and also in cells in normal solution at depolarized potentials, conditions which would tend to cause an increased level of internal calcium. Application of acetylcholine sometimes induced a brief potentiation of these transient outward currents in both frequency and amplitude. This potentiation preceded the onset of the inward current, and was consistently followed by long-lasting inhibition of the transient outward currents (Fig. 2a). These brief currents may represent the activation in response to transient increases in intracellular calcium of Ca^{2+} -activated K^+ channels which we have identified in these cells¹²; the transient currents reversed at the K^+ equilibrium potential (Fig. 2a) were blocked, by external Ba^{2+} ions (2-10 mM) and were not seen if Cs^+ replaced K^+ in the pipette or if the concentration of EGTA in the pipette was raised to 10 mM. These currents were activated by caffeine (5 mM), which is known to release intracellular stores of Ca^{2+} in muscle¹³. Fluctuations in intracellular calcium concentration ($[Ca^{2+}]_i$) that might stimulate such currents have been measured directly in cardiac cells¹⁴, and similar transient potassium currents caused by fluctuations in $[Ca^{2+}]_i$ have been observed in neurones¹⁵.

A biochemical mediator within the cell may be important in triggering the inward current produced by muscarinic receptor activation, as the long latency of this response suggests a rather indirect coupling between receptor and channels. The latency can no longer be dismissed as being due to a long extracellular diffusion pathway, as in our system we know the cells must have clean, naked membranes to obtain gigaohm seals with our recording pipette. (A diffusion distance of 10 μm would induce a nicotinic response at the neuromuscular junction in less than 10 ms¹⁶. A rise in $[Ca^{2+}]_i$ does not seem to be a possible mechanism (as in neurones¹⁷ and the heart¹⁸), because caffeine did not stimulate an inward current.

In conclusion, we have succeeded in dissecting some of the complex membrane events that underlie the excitatory muscarinic action of acetylcholine in mammalian smooth muscle. The major effect of acetylcholine was to stimulate a voltage-dependent inward current that leads to depolarization of the cell and initiates action potential discharge. This current was large at the potassium equilibrium potential and was a non-selective increase in permeability, probably to cations, as has been described for whole smooth muscle tissues^{1,2,19}. In addition, acetylcholine causes a long-lasting inhibition of spontaneous outward current transients, which are probably caused by the transient opening of Ca^{2+} -activated K^+ channels. The two types of channel modulation that we have observed are sufficient to explain the membrane responses of whole tissues of mammalian visceral smooth muscle to acetylcholine.

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Inositol 1,4,5-trisphosphate induces calcium release from sarcoplasmic reticulum of skeletal muscle

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The sarcoplasmic reticulum of skeletal muscle is a specialized form of endoplasmic reticulum¹ that controls myoplasmic calcium concentration and, therefore, the contraction-relaxation cycle². Ultrastructural studies³ have shown that the sarcoplasmic reticulum is a continuous but heterogeneous membranous network composed of longitudinal tubules that surround myofibrils and terminal cisternae. These cisternae are junctionally associated, via bridging structures called 'feet'⁴, with sarcolemmal invaginations (the transverse tubules) to form the triadic junction⁴. Following transverse tubule depolarization, a signal, transmitted along the triadic junction, triggers Ca^{2+} release from terminal cisternae^{5,6}, but the mechanism of this coupling is still unknown⁷. Inositol 1,4,5-trisphosphate ($\text{Ins}(1,4,5)\text{P}_3$) has recently been shown to mobilize Ca^{2+} from intracellular stores, referable to endoplasmic reticulum, in a variety of cell types (see ref. 8 for review), including smooth muscle cells of the porcine coronary artery⁹ and canine cardiac muscle cells¹⁰. Here we show that $\text{Ins}(1,4,5)\text{P}_3$ (1) releases Ca^{2+} from isolated, purified sarcoplasmic reticulum fractions of rabbit fast-twitch skeletal muscle, the effect being more pronounced on a fraction of terminal cisternae that contains morphologically intact feet structures¹¹; and (2) elicits isometric force development in chemically skinned muscle fibres.

Ca^{2+} uptake by and Ca^{2+} release from isolated sarcoplasmic reticulum (SR) fractions were measured with a Ca^{2+} -sensitive electrode. Figure 1a shows that terminal cisternae (TC) accumulated 43 nmol of Ca^{2+} per mg of protein and then (dashed line) reached a slightly lower steady state¹². Addition of 25 μM $\text{Ins}(1,4,5)\text{P}_3$ (continuous line) promoted Ca^{2+} release, and a new, much lower steady state was attained. Following a biphasic pattern, approximately 50% of the accumulated Ca^{2+} was released within 5 min and then slowly re-accumulated. The effect of $\text{Ins}(1,4,5)\text{P}_3$ was specific since neither inositol 1,4-bisphosphate ($\text{Ins}(1,4)\text{P}_2$) nor inositol 4,5-bisphosphate ($\text{Ins}(4,5)\text{P}_2$) caused Ca^{2+} release from either TC fractions or fractions of longitudinal tubules (LSR), when tested at 25 μM (Table 1).

Figure 1b shows the relationship between the extent of Ca^{2+} release and $\text{Ins}(1,4,5)\text{P}_3$ concentration. Half-maximal stimulation of Ca^{2+} release was obtained at 4–5 μM $\text{Ins}(1,4,5)\text{P}_3$ for both TC and LSR fractions. $\text{Ins}(1,4,5)\text{P}_3$ was more effective on TC than on LSR, where only 20% of the accumulated Ca^{2+} was released. The partial sensitivity of LSR to $\text{Ins}(1,4,5)\text{P}_3$ is due, at least in part, to the contamination of this fraction by about 20% TC vesicles (compare with ref. 11), as indicated by the effect of ruthenium red, an inhibitor of Ca^{2+} release from TC¹³⁻¹⁵ but not from LSR^{15,16} fractions. Ruthenium red at 20 μM almost completely blocked $\text{Ins}(1,4,5)\text{P}_3$ -induced Ca^{2+} release from TC, but produced a decrease of only 50% in release from LSR

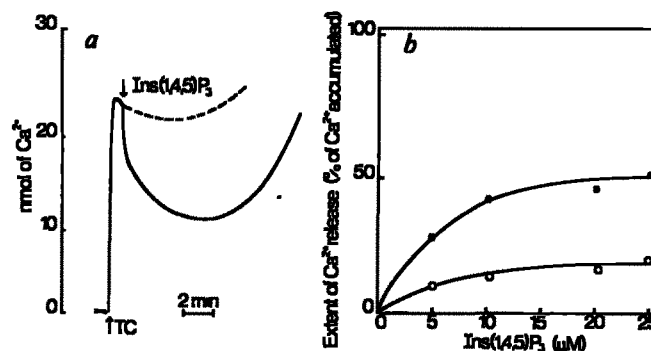


Fig. 1 $\text{Ins}(1,4,5)\text{P}_3$ promotes Ca^{2+} release from isolated SR fractions. *a*, After peak Ca^{2+} accumulation, there was a small, basal Ca^{2+} release (dashed line). Addition of 25 μM $\text{Ins}(1,4,5)\text{P}_3$ (continuous line) promoted a large Ca^{2+} release from TC, and a new lower steady state was attained. The rate of Ca^{2+} release induced by $\text{Ins}(1,4,5)\text{P}_3$ (fast phase) could not be determined accurately because of the inherent slow response of the Ca^{2+} electrode. As a lower limit, we calculated a rate of 1 nmol of Ca^{2+} per s per mg of TC protein, that is, three orders of magnitude lower than that estimated *in vivo*³¹ $\text{Ins}(1,4,5)\text{P}_3$ by itself produced only a small increase of medium Ca^{2+} that accounted for about 4% of the effect shown. *b*, A summary of data obtained from several experiments as shown in *a*, using either LSR (O) or TC (●) fractions. The extent of $\text{Ins}(1,4,5)\text{P}_3$ -induced Ca^{2+} release was calculated as the difference between total (+ $\text{Ins}(1,4,5)\text{P}_3$) and basal Ca^{2+} release at steady state, and was expressed as percentage of Ca^{2+} accumulated by the SR. Similar results were obtained using three different SR preparations.

Methods. SR fractions were isolated from rabbit fast-twitch skeletal muscles and purified by differential and density gradient centrifugation^{11,32}. R2 (LSR) and R4 (TC) fractions were resuspended in 0.3 M sucrose, 5 mM imidazole pH 7.4 and stored at -70°C until used. Protein concentration was determined by the Lowry method³³, using bovine serum albumin as a standard. $\text{Ins}(1,4,5)\text{P}_3$ was prepared by incubating human erythrocyte ghosts at 37°C for 15 min with 2 mM CaCl_2 , followed by Dowex-formate column separation, salt removal with 2 M LiCl and three consecutive extractions with ethanol^{24,34}. Ca^{2+} uptake and Ca^{2+} release were continuously monitored with a Ca^{2+} -sensitive electrode (G. Moller, Zurich). The assay was carried out at room temperature ($22-24^\circ\text{C}$) in a medium containing, in a final volume of 4 ml, 20 mM Tris-Cl pH 6.8, 0.1 M KCl, 5 mM MgSO_4 , 2.5 mM ATP, 5 mM $\text{Na}_2\text{-phosphocreatine}$ and 80 μg creatine kinase. Several pulses of 10 nmol CaCl_2 were sequentially added to calibrate the response of the Ca^{2+} electrode, and the reaction was started with either 600 μg of TC or 300 μg of LSR protein, when medium-free Ca^{2+} was 4 μM . LSR and TC fractions accumulated 102 ± 9 and 40 ± 5 nmol of Ca^{2+} per mg of protein, respectively (mean $\pm$ s.d., $n = 5$).

(Table 1). The small ruthenium red-insensitive $\text{Ins}(1,4,5)\text{P}_3$ -induced Ca^{2+} release appears to be a LSR characteristic (Table 1). Thus, our results suggest that $\text{Ins}(1,4,5)\text{P}_3$ acts preferentially on junctional SR (TC) fractions. Interestingly, Berridge and Irvine⁸ have postulated a subspecialization for endoplasmic reticulum of other cell types in which only regions lying close to the plasma membrane might be sensitive to $\text{Ins}(1,4,5)\text{P}_3$.

The ability of $\text{Ins}(1,4,5)\text{P}_3$ to release Ca^{2+} from the SR was further investigated using a chemically skinned fibre preparation¹⁷. Ca^{2+} release from the SR was measured indirectly by monitoring isometric force development¹⁷. Segments of single fibres (average mean diameter 50 μm) were exposed to Ca^{2+} loading solution (free $\text{Ca}^{2+} = 0.40 \mu\text{M}$) for 30 s, so that the SR could accumulate Ca^{2+} , rinsed in EGTA-free solution and then challenged with $\text{Ins}(1,4,5)\text{P}_3$ that elicited transient force development (Fig. 2a). At low $\text{Ins}(1,4,5)\text{P}_3$ concentrations (1 μM , upper trace in Fig. 2a), the time course of force development was biphasic, that is, a slow phase turned into a faster one after approximately 30 s. An S-shaped time course suggests that $\Delta[\text{Ca}^{2+}]/\Delta t$ in the intermyofibrillar space was very small¹⁸ after stimulation. At higher $\text{Ins}(1,4,5)\text{P}_3$ concentrations, (20 μM , lower trace in Fig. 2a), force development was monophasic. These results imply that $\text{Ins}(1,4,5)\text{P}_3$ was able to release Ca^{2+}

from TC, which are the only Ca^{2+} storage compartments of the SR of skeletal muscle fibres^{6,19}. At 20 μM $\text{Ins}(1,4,5)\text{P}_3$, $t_{1/2}$ to peak tension was 3 s and rate of tension rise, normalized against that obtained in the presence of 10 mM caffeine, a drug known to cause Ca^{2+} release from TC of isolated fractions²⁰ and of intact²¹ and skinned²² fibres, was 0.58. The slow rate of force development might be determined by the rate of $\text{Ins}(1,4,5)\text{P}_3$ diffusion throughout the cross-section of the fibre, and by the extent of $\text{Ins}(1,4,5)\text{P}_3$ hydrolysis (see below). Figure 2b shows a plot of relative rate of tension rise versus $\text{Ins}(1,4,5)\text{P}_3$ concentration. Half-maximal rate was obtained at 3 μM . $\text{Ins}(1,4,5)\text{P}_3$ action depended critically on Ca^{2+} loading by the SR of skinned fibres. The effect of 10 μM $\text{Ins}(1,4,5)\text{P}_3$ on the rate of tension rise was decreased by 70% when loading time was reduced from 30 to 15 s or was abolished if the free Ca^{2+} of the loading solution was decreased from 0.4 to 0.16 μM .

As with isolated SR fractions, the ability to release Ca^{2+} appears to be specific for $\text{Ins}(1,4,5)\text{P}_3$, since 20 μM of either $\text{Ins}(1,4)\text{P}_2$ or $\text{Ins}(4,5)\text{P}_2$ had a minimal effect on force development in skinned fibres, that is 3–6% of that obtained with the same amount of $\text{Ins}(1,4,5)\text{P}_3$ (not shown). Furthermore, $\text{Ins}(1,4,5)\text{P}_3$ -induced force development was completely abolished by 20 μM ruthenium red (not shown). Ruthenium red inhibits Ca^{2+} release from the SR of chemically skinned fibres (G.S. and P.V., in preparation) as well as from isolated TC fractions.

The proposed role of $\text{Ins}(1,4,5)\text{P}_3$ as a second messenger for releasing Ca^{2+} from intracellular stores⁸ requires: first, substrates (phosphatidylinositol 4,5-bisphosphate, $\text{PtdIns}(4,5)\text{P}_2$), specific hydrolases ($\text{PtdIns}(4,5)\text{P}_2$ phosphodiesterase or phospholipase C) at the surface membrane level, and inactivating enzymes (inositol trisphosphatase); second, specificity for endoplasmic reticulum receptors; and third, a polyphosphoinositide breakdown rate compatible with cellular response times.

The data reported here are consistent with the possibility that $\text{Ins}(1,4,5)\text{P}_3$ is the specific chemical transmitter in the triadic junction where signal transduction for muscle activation occurs. In fact: (1) $\text{Ins}(1,4,5)\text{P}_3$ -induced Ca^{2+} release was preferentially displayed by TC fractions, which are enriched in junctional SR membranes, and was antagonized by ruthenium red, a blocker of TC Ca^{2+} channels¹³; (2) $\text{Ins}(1,4,5)\text{P}_3$ evoked Ca^{2+} release from TC of skinned muscle fibres; and (3) $\text{Ins}(1,4,5)\text{P}_3$ was preferred over $\text{Ins}(1,4)\text{P}_2$ and $\text{Ins}(4,5)\text{P}_2$. A simplified model for transverse tubule (TT)–TC coupling may be outlined as follows. After TT depolarization, $\text{Ins}(1,4,5)\text{P}_3$ is produced at the myoplasmic leaflet of the TT membrane and released into the triadic junction; $\text{Ins}(1,4,5)\text{P}_3$ -sensitive Ca^{2+} channels, localized in junctional SR (TC) membranes, open and myoplasmic free Ca^{2+} rises. Alternatively, $\text{Ins}(1,4,5)\text{P}_3$ may evoke a small Ca^{2+}

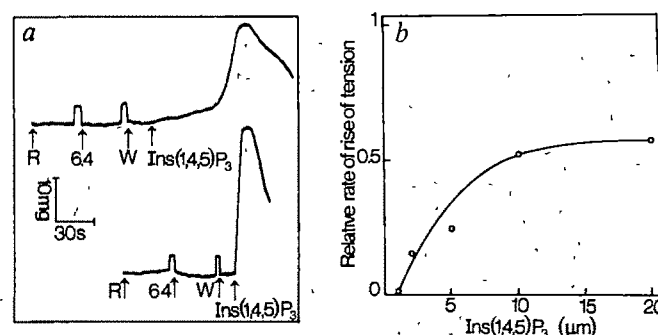


Fig. 2 $\text{Ins}(1,4,5)\text{P}_3$ elicits isometric force development in chemically skinned muscle fibres. *a*, Upper and lower traces show the effect of 1 and 20 μM $\text{Ins}(1,4,5)\text{P}_3$, respectively. *b*, A summary of data from several experiments as shown in *a*. Rate of tension rise, at specified $\text{Ins}(1,4,5)\text{P}_3$ concentration, was normalized against that obtained with 10 mM caffeine. When rates were biphasic, faster rates were considered. Variability was observed in the response to 20 μM $\text{Ins}(1,4,5)\text{P}_3$: 10 fibres were fully responsive, 4 fibres were partially responsive (~20% of the effect shown in *b*) and 3 fibres were not responsive at all. Such differences can be tentatively ascribed to variations in (1) $\text{Ins}(1,4,5)\text{P}_3$ hydrolysis (which should be already low at 0.1 mM free Mg^{2+})^{23,24}; (2) Ca^{2+} loading by the fibres and (3) fibre diameter and relative diffusion times into the fibre. In some experiments, we used $\text{Ins}(1,4,5)\text{P}_3$ derived from ox brain $\text{PtdIns}(4,5)\text{P}_2$ (ref. 30) and results were comparable.

Methods. Rabbit adductor muscle bundles were chemically skinned^{17,35} and stored at -20°C in a solution containing 50% glycerol, 5 mM $\text{K}_2\text{-EGTA}$, 0.17 M K-propionate pH 7.0, 2.5 mM $\text{K}_2\text{-Na}_2\text{-ATP}$, 2.5 mM Mg-propionate and 10 mM imidazole propionate. For isometric force measurements, segments of single fibres were positioned between two clamps, one of them attached to a strain gauge, and stretched to 130% of the slack length³⁵. After 30–45 s in solution R (5 mM $\text{K}_2\text{-EGTA}$, 0.17 M K-propionate , 2.5 mM Mg-propionate , 5 mM $\text{K}_2\text{-Na}_2\text{-ATP}$ and 10 mM imidazole propionate pH 7.0) fibres were allowed to accumulate Ca^{2+} for 30 s in a $p\text{Ca}$ 6.4 loading solution (0.17 M K-propionate , 2.5 mM Mg-propionate , 5 mM $\text{K}_2\text{-Na}_2\text{-ATP}$, 2.5 mM CaCl_2 , 5 mM $\text{K}_2\text{-EGTA}$ and 10 mM imidazole propionate pH 7.0), rinsed for 20–30 s in solution W (solution R without EGTA), and then challenged with $\text{Ins}(1,4,5)\text{P}_3$. Free Mg^{2+} (kept constant at 0.09 mM) and free Ca^{2+} (0.40 μM) were determined using association constants given in Orentlicher *et al.*³⁶ All experiments were carried out at room temperature ($22\text{--}24^\circ\text{C}$) and ionic strength 0.2 M.

efflux which, in turn, triggers a massive Ca^{2+} release by opening TC Ca^{2+} -gated Ca^{2+} channels¹³. The relevance of this model to *in vivo* Ca^{2+} release could not be assessed fully in the skinned fibre preparation because of an experimental constraint that cannot be overcome, that is, exogenous $\text{Ins}(1,4,5)\text{P}_3$ must be added to the bathing solution from where it diffuses into the fibre. Interestingly, $\text{Ins}(1,4,5)\text{P}_3$ triggered Ca^{2+} release from the SR of mechanically skinned frog skeletal muscle fibres²³, and $\text{Ins}(1,4,5)\text{P}_3$ was more effective²³ if its hydrolysis was inhibited by either low free Mg^{2+} (ref. 24) or 2, 3-diphospho-D-glycerate²⁴.

The proposed model is still highly speculative and several crucial questions remain unanswered: does $\text{PtdIns}(4,5)\text{P}_2$ phosphodiesterase exist in TT membranes and, more importantly, does polyphosphoinositide breakdown take place during muscle activation? In this respect we note that Novotny *et al.*²⁵ have shown that K^+ depolarization increased ^{32}P -labelling of phosphatidylinositol in frog sartorius muscles, and that the phosphatidylinositol response is caused by K^+ depolarization and not by the ensuing transient Ca^{2+} release from the SR. Moreover, preliminary data obtained by us indicate that water-soluble inositol phosphates could be extracted from rat diaphragm muscle and that ^3H -*myo*-inositol incorporation into membrane phospholipids increased following a 5-s tetanus. Finally, in frog sartorius and semitendinosus muscles, a short-pulse tetanus followed by rapid freezing²⁶ increased the level of phosphatidylinositol, $\text{Ins}(4,5)\text{P}_2$ and $\text{Ins}(1,4,5)\text{P}_3$ to levels three to four times those in controls (J. Vergava and R. Y. Tsien, personal communication). Taken together, these observations suggest that

Table 1 Effect of ruthenium red on $\text{Ins}(1,4,5)\text{P}_3$ -induced Ca^{2+} release from isolated SR fractions, and effect of $\text{Ins}(1,4)\text{P}_2$ and $\text{Ins}(4,5)\text{P}_2$

Additions	Extent of Ca^{2+} release (% of Ca^{2+} accumulated)	
	LSR	TC
$\text{Ins}(1,4,5)\text{P}_3$ (25 μM)	17	47
$\text{Ins}(1,4,5)\text{P}_3$ (25 μM) plus ruthenium red (20 μM)	7	8
$\text{Ins}(1,4)\text{P}_2$ (25 μM)	2	1
$\text{Ins}(4,5)\text{P}_2$ (25 μM)	3	1

Ca^{2+} uptake and Ca^{2+} release were measured as described in Fig. 1 legend. $\text{Ins}(1,4)\text{P}_2$ and $\text{Ins}(4,5)\text{P}_2$ were prepared by alkaline hydrolysis of ox brain $\text{PtdIns}(4,5)\text{P}_2$ (ref. 30). With no additions there was a basal Ca^{2+} release that corresponded to the slightly lower steady state attained after peak Ca^{2+} accumulation (compare Fig. 1a, dashed line), and accounted for approximately 4% and 8% of the Ca^{2+} accumulated in LSR and TC fractions, respectively. The extent of $\text{Ins}(1,4,5)\text{P}_3$ induced Ca^{2+} release was calculated as the difference between total and basal Ca^{2+} release (see Fig. 1). Data are from a typical experiment in which duplicate determinations were performed.

polyphosphoinositide metabolism may be causally related to muscle activation.

Another fundamental question is whether the phosphoinositide breakdown rate is fast enough (in the millisecond range) to exert a physiological role in skeletal muscle contraction. This question was not directly addressed. However, the recent findings^{27,28} that $\text{PtdIns}(4,5)\text{P}_2$ breakdown is involved in phototransduction, a quite complex and rapid event²⁹, encourage us not to rule out *a priori* such a mechanism for muscle activation.

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Mapping of general anaesthetic target sites provides a molecular basis for cutoff effects

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A longstanding and unresolved problem in general anaesthesia is the so-called 'cutoff' effect; as one ascends a homologous series of anaesthetic agents, the potencies progressively increase with anaesthetic size but then, rather suddenly, anaesthetic potency disappears^{1–5}. Curiously, this cutoff in potency occurs at very different points in different series. Various explanations have been offered^{5,6}, usually based on the notion that lipid bilayers are the primary target sites in general anaesthesia^{3,4,7}. However, accumulating evidence now suggests that proteins are the primary sites of action^{8–14}. Here we demonstrate cutoff effects for the anaesthetic inhibition of a soluble protein (firefly luciferase) which mirror those found for general anaesthesia, and we describe how the molecular architecture of the binding site accounts for the different cutoffs in the different homologous series. We show that this

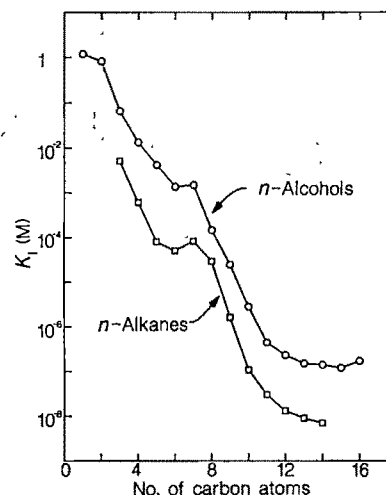


Fig. 1 Inhibition constants K_i for the homologous series of *n*-alcohols and *n*-alkanes acting on the enzyme luciferase. Firefly luciferase was purified, its activity assayed and the inhibition constants for competition with the natural substrate luciferin determined as described previously¹⁴. Standard errors in the K_i values were $\leq 20\%$. As before, the data were analysed using a simple binding model¹⁴ in which *N* anaesthetic molecules bind to the enzyme with equal affinities but only one molecule is sufficient to cause inhibition. We find that the break between *N* = 2 and *N* = 1 occurs for the alcohols between *C*₇ and *C*₈. For the alkanes, we confirmed that this break occurs between *C*₆ and *C*₁₀. However, the low activities of the intervening members meant that the exact point could not be ascertained; we took it as being the same as for the alcohols. For the largest molecules with *N* = 2, it seems likely that the two molecules bind with different affinities, although our data were not sufficiently precise to detect this. In such cases, the K_i values we have determined represent weighted averages. The alcohols *C*₁₁–*C*₁₆ were added as ethanolic solutions, such that the final ethanol concentration never exceeded 5% of the K_i for ethanol; the controls nonetheless contained the appropriate concentration of ethanol. Solutions containing the gaseous alkanes *C*₃ and *C*₄ were prepared by bubbling the gases through scintered glass, results obtained after 1 day were identical to those obtained after 2 days of bubbling. The final concentrations were calculated using the mole fraction solubilities given in ref. 18. Saturated solutions of alkanes *C*₅–*C*₁₃ were prepared by prolonged and vigorous shaking with small (~20 ml) volumes of buffer. In addition, alkanes *C*₈–*C*₁₄ were added as ethanolic solutions such that their final concentrations were only about five times in excess of their saturated solutions. Results obtained using these two methods gave excellent agreement. Values for the saturated aqueous solutions of these alkanes were calculated using the straight line relationship shown in Fig. 2b. For each anaesthetic, the concentration of enzyme was always kept well below the lowest concentration of anaesthetic used. The alcohols and alkanes were the purest grades available from Sigma or BDH.

behaviour is a natural consequence of anaesthetics binding to an amphiphilic protein pocket of circumscribed dimensions. When general anaesthetic target sites in animals and the luciferase protein are mapped out using the fine details of the potency data, remarkable similarities are revealed. Our results thus suggest that the target sites in general anaesthesia are amphiphilic pockets on proteins.

The best-characterized examples of homologous series which display cutoffs in anaesthetic potency are the *n*-alcohols and *n*-alkanes. General anaesthetic activity disappears at different points in these two series; the alcohols^{1,4} cut off at about *C*₁₃ while the alkanes^{2,5} cut off between *C*₆ and *C*₁₀, the exact point being species-dependent. To elucidate the molecular basis for these observations, we have determined the inhibition constants for homologous series of both alcohols and alkanes acting on a pure soluble protein. This protein, firefly luciferase, is competitively inhibited by a wide range of general anaesthetics at concentrations which are essentially identical to those which produce general anaesthesia in animals¹⁴.

The inhibition constants K_i for the homologous series of

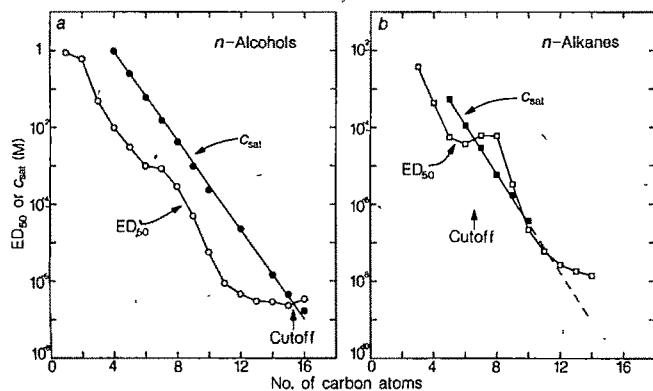


Fig. 2 The cutoff effect for the homologous series of *n*-alcohols (a) and *n*-alkanes (b). The ED_{50} values are the concentrations of anaesthetics which inhibited luciferase activity by 50% when the concentration of the substrate luciferin was equal to the K_m of the uninhibited enzyme ($14 \mu M$). Standard errors in the ED_{50} values were $\leq 20\%$. The c_{sat} values are the maximum possible aqueous concentrations (the solubilities) of the anaesthetics. When it was not possible to achieve 50% inhibition experimentally, ED_{50} values were estimated by extrapolation from the maximum attainable inhibitions. The c_{sat} values for the alcohols and alkanes are those compiled in ref. 19. The c_{sat} straight lines are drawn according to the equations given in ref. 19; for the alkanes we have assumed¹⁹ that the line can be extrapolated to C_{14} . Arrows indicate the positions of the abrupt cutoffs in potency.

n-alcohols and *n*-alkanes acting on firefly luciferase are plotted against the number of carbon atoms in Fig. 1. The two curves display some expected but also some surprising features. As might be expected for binding to a hydrophobic pocket, the alkanes bind more tightly than their corresponding alcohols, and the addition of a hydrophobic methylene group ($-\text{CH}_2-$) to either type of molecule tends to increase the binding affinity (that is, to reduce K_i). Interestingly, however, the alkanes only bind 5–50 times more tightly than their corresponding alcohols, very much less than the 10,000-fold factor for partitioning between water and a purely hydrophobic solvent such as hexadecane¹⁵. Evidently, the anaesthetic-binding site on luciferase behaves as if it is amphiphilic, containing polar as well as apolar parts. This might be expected of a binding site which presumably extends to the water/protein surface. What is more surprising is that there are two regions in each curve of Fig. 1, at about C_6 and after about C_{12} , where the K_i values level off. In other words, there are two regions in which making an anaesthetic molecule larger and more hydrophobic does not change the free energy of binding.

What molecular interpretation can be placed on these results? We have shown previously¹⁴ that the anaesthetic-binding site on luciferase has a volume roughly equal to that of two hexanol molecules and that for agents larger than heptanol only a single molecule can be accommodated. Thus, at around C_6 , when two molecules bind, the site is becoming fully occupied, so that additional CH_2 groups can no longer bind within the pocket but must remain in water, where they can make little or no contribution to the overall binding energy. The K_i curves therefore tend to level off in this region. Between C_8 and C_{12} , where only one molecule binds, additional CH_2 groups are no longer forced into a polar environment and thus contribute strongly to binding. After C_{12} , a single molecule is now large enough to fill the binding site so that additional CH_2 groups remain in water and the K_i curves level off once again. An alternative explanation for the levelling off at about C_6 is that the hydrocarbon chains encounter packing constraints or even a polar region near the bottom of the pocket, so that CH_2 groups in this region bind weakly. (Such a polar region has previously been postulated¹⁶ to account for the relatively weak binding of hydrocarbons to bovine serum albumin.) Although the details of the molecular interpretation must await future work, the levelling-off of the K_i curves can be accounted for by the anaesthetics binding to

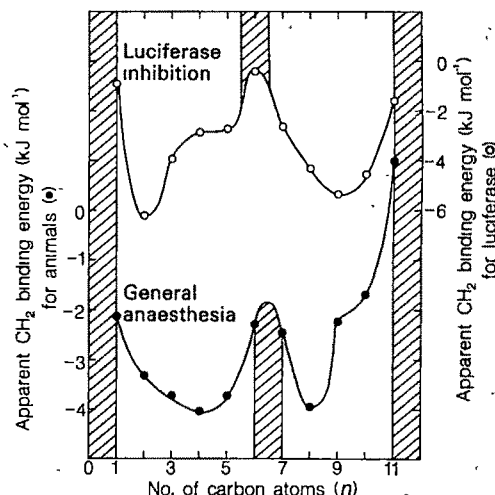


Fig. 3 Corresponding molecular features in the target sites in general anaesthesia and in the anaesthetic-binding pocket on the luciferase protein. The apparent changes in binding energy for a methylene group binding to luciferase (top) and target sites for general anaesthesia (bottom) are plotted as a function of chain length (n). Note the different scales in the vertical direction. Each point was calculated using the difference in thermodynamic free energy, $\Delta\Delta G^\ominus = -RT \ln[(ED_{50})_n/(ED_{50})_{n+1}]$ and alcohol ED_{50} values. The luciferase ED_{50} values are those plotted in Fig. 2a while those for general anaesthesia in animals (tadpoles) are taken from Tables 5 ($n = 1-8$) and 4 ($n = 9-12$) of ref. 20. True binding energies for animal target sites can only be calculated using inhibition constants which are, of course, unknown. So that a fair comparison between the data for general anaesthesia and luciferase inhibition could be made, we have used ED_{50} values throughout. The cross-hatched areas represent regions where methylene (CH_2) groups bind relatively weakly. A molecular interpretation of these features is given in the text.

an amphiphilic pocket of circumscribed dimensions.

To make a direct comparison with known ED_{50} concentrations for general anaesthesia, we determined luciferase ED_{50} concentrations for all of the above agents. (An ED_{50} concentration is one which produces a 50% effect, either anaesthetizing 50% of a population of animals or reducing luciferase activity by 50%.) Since alcohols larger than C_{11} all bind to the protein with roughly equal affinities (see Fig. 1), it might be expected that all of these large molecules would be equally effective at inhibiting enzyme activity. However, while C_{12} , for example, is among the most potent alcohols, we found that C_{16} was not an effective inhibitor. The resolution of this paradox is clear from the data shown in Fig. 2a, where we have plotted alcohol ED_{50} concentrations together with the corresponding maximum aqueous solubilities (c_{sat}). It can be seen that C_{16} is ineffective simply because its maximum achievable aqueous concentration lies well below that necessary to halve the enzyme activity.

Because of the similarities between the alkane and alcohol K_i curves in Fig. 1, one might suppose that the ED_{50} cutoff points for the two series would occur at similar places. However, as observed for general anaesthesia, we found that the alkanes cut off much earlier. The reason for this cutoff is clear from the data in Fig. 2b: the c_{sat} line intercepts the alkane ED_{50} curve between C_6 and C_7 . After C_8 , the site is occupied by only a single molecule and binding again increases such that C_{10} and C_{11} become active, but then, once more, the finite size of the pocket comes into play. (Although this recovery of activity has not, to our knowledge, been looked for in animals, it might well be peculiar to luciferase.)

Thus, for inhibition of luciferase activity, alcohol and alkane cutoffs appear at very different points. The alcohol cutoff at C_{16} is a consequence of the levelling-off of the ED_{50} curve (Fig. 2a) beyond about C_{12} , a result of the circumscribed dimensions of the binding pocket. The alkane cutoff at C_7 , on the other hand, results from the unexpected levelling-off of the ED_{50} curve (Fig. 2b) at about C_6 . In practical terms, the cutoffs occur not because

the anaesthetic molecules bind weakly to the protein but because the maximum aqueous solubilities of the inactive agents lie below the concentrations needed to half-inhibit activity. Our observations provide a plausible basis for interpreting cutoffs in anaesthetic potencies in animals. For tadpoles^{1,4}, the alcohol cutoff occurs slightly earlier (at C_{13} instead of C_{16}), and this may reflect a somewhat smaller volume for general anaesthetic target sites in animals. The alkane cutoffs for different animals^{2,5} occur between C_6 and C_{10} , similar to our value of C_7 .

In lipid theories, alcohol⁴ and alkane⁷ cutoffs have been accounted for in terms of an abrupt decrease in absorption into lipid bilayers. Very recently, however, it has been shown¹⁷ that partition coefficients for *n*-alkyltrimethylammonium ions continue to increase, at least up to C_{18} , with no suggestion of a sudden cutoff in absorption. It seems unlikely, therefore, that potency cutoffs in other amphiphiles, such as the *n*-alcohols, can be explained in terms of bilayer solubility.

Finally, in Fig. 3 we have used alcohol ED_{50} data to make a direct comparison between anaesthetics binding to the luciferase protein and to the target sites involved in general anaesthesia, by plotting the apparent changes in CH_2 binding energies as the alcohol molecule increases in length. The cross-hatched areas indicate those regions on the luciferase and central nervous system target sites where CH_2 groups bind relatively weakly. The remarkable similarity between the data for general anaesthesia and for inhibition of the protein luciferase suggests that corresponding molecular features may be present in the target

sites for general anaesthesia in animals.

Although these target sites in animals have yet to be identified, a pocket of circumscribed dimensions and amphiphilic nature might be expected for a protein site which must selectively bind and orient relatively apolar ligands containing at least one polar group.

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Tissue-specific modulation of a set of related cell surface antigens in *Drosophila*

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To be integrated correctly into the developing organism, cells need to interact with an environment that includes neighbouring cells, the extracellular matrix and soluble factors. These interactions probably require specific recognition events mediated by cell-surface molecules, and evidence has accumulated for the presence of such molecules in several organisms (see, for example, refs 1–6). In *Drosophila*, a family of cell-surface glycoprotein complexes, the position-specific (PS) antigens, may be involved in such processes^{7–11}. PS antigens show spatially restricted distributions on imaginal disks that vary during development; their expression on any particular cell correlates with the position of that cell in the developing disk epithelium rather than with its ultimate differentiation. In contrast to the situation in disks, however, immunofluorescence revealed few regional restrictions of PS-antigen expression on other larval tissues^{7,11}. If the PS antigens have a general role in morphogenesis, then widespread regional variation in their distributions might be expected. We now report that, by using polyacrylamide gel electrophoresis (PAGE), a technique that gives higher resolution than immunofluorescence, we detect an increase in both the concentration and the diversity of the complexes during embryogenesis, beginning at gastrulation. Further, each larval and adult tissue examined carries its own characteristic set of complexes. This tissue- and region-specific diversity is achieved by combining different members of a set of related components with a glycoprotein common to all the complexes.

Monoclonal antibodies against the PS1 and PS2 antigens were defined by their preferential binding to cells of the dorsal and ventral compartments, respectively, of the mature wing imaginal disk^{7,8}. The antibodies precipitate different but related complexes of glycoproteins from disk lysates (refs 8, 9; lanes *a* in Fig. 1). Each antibody recognizes components unique to its own complex: glycoprotein 116 (gp116) and gp120 in the case of

PS1 antibodies; gp125 for the PS2 antibodies⁹. A third class of antibodies, PS3, recognize determinants on a gp110 component common to the complexes. PS3 antibodies thus precipitate both the PS1 and PS2 complexes together with at least one further complex containing a gp92 doublet.

To investigate the expression of the antigens on tissues other than imaginal disk, we analysed the composition of embryonic antigens at different stages of development (see Fig. 1 legend for a description of the timing of the main events of embryogenesis) and on different larval and adult tissues to determine whether we could detect variations or restricted distributions not revealed by immunofluorescence staining. Lysates of embryos and of first (24 and 28 h)- and second (52 h)-instar larvae were extracted sequentially, first with PS1, then with PS2 and finally with PS3 antibodies (see Fig. 1 legend) and the antigen components were separated by SDS-PAGE. The main findings, shown in Fig. 1, can be summarized as follows.

(1) The PS complexes are present only at low concentrations until after the onset of gastrulation (which begins ~3.5 h after egg laying, shortly after the formation of the cellular blastoderm). In Fig. 1, only the 1 h and 4 h time points are shown but sampling at other times within this period confirms the observation. From about 4 to 6 h, all PS antigens show a dramatic increase of both amount and complexity.

(2) PS1 and PS2 antibodies extract discrete subsets of the total PS complexes at each stage, leaving behind others which can be extracted subsequently by PS3 antibodies.

(3) Different components appear and reach their maximum levels at different times and several appear only transiently or show fluctuations in their level, for example, the PS1 component of relative molecular mass (M_r) 120,000 (120K), or the PS3 doublet at 92K.

(4) Several components found in embryonic PS antigens do not occur in the disk antigens (lanes *a* of Fig. 1). Thus, each antibody extracts different complexes from different lysates. Moreover, the large number of components often seen in the antigen extracted from embryos by any given antibody suggests that each antibody may be precipitating several different complexes, each expressing a molecule carrying the determinant recognized by that antibody, but containing different components attached to it. Extrapolating from what we know of the spatially restricted distribution of PS antigens on imaginal disks, it seemed likely that the additional complexes in the embryo

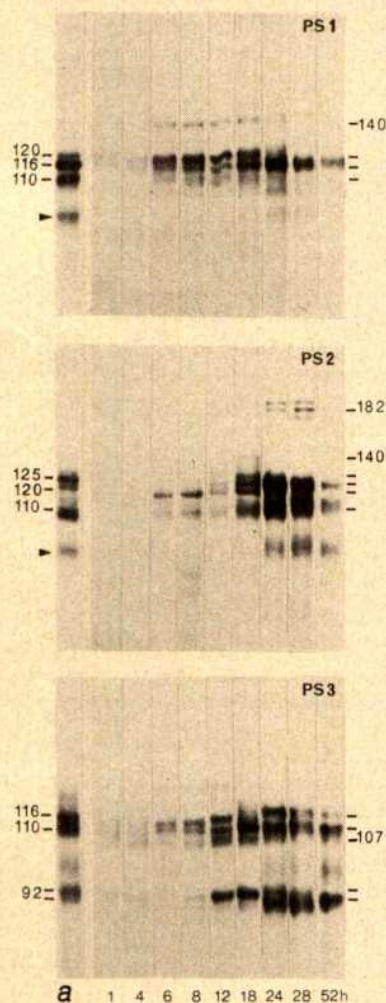
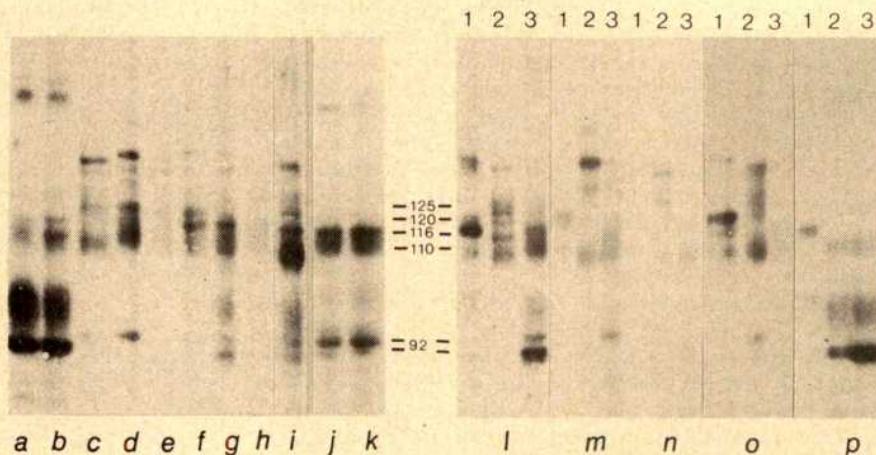


Fig. 1 Embryonic and early larval PS antigens. Embryos or larvae of the ages shown were lysed, the lysates extracted in turn with PS1, PS2 and finally PS3 antibodies, and the PS-antigen components resolved by SDS-PAGE. Preliminary experiments showed that the PS1 and PS2 antibody levels were sufficient to remove all antigens recognized by those antibodies. The overall pattern of components and the timing of their appearance is reproducible from experiment to experiment. Lanes *a* show, for comparison, the components of PS1, PS2 and PS3 antigens extracted sequentially from an imaginal disk lysate. The band indicated by an arrowhead at ~100K is a breakdown product of gp110 (ref. 9).

Methods. Eggs were collected from *Drosophila melanogaster* flies (Oregon R strain) over 1-h laying periods, dechorionated and fractured by sonication at the times shown (=time of development $\pm$ 30 min), and proteins were solubilized in 10 vol. of a non-ionic detergent buffer containing 20 mM Tris-HCl pH 8.1, 100 mM NaCl, 1 mM each of $MgCl_2$ and $CaCl_2$, 0.2% each of Nonidet-P40 and Triton X-100, 1% kallikrein inactivator and 1 mM phenylmethylsulphonyl fluoride. Equal volumes (50 μ l) of packed embryos or larvae were used at each time point. Each lysate was extracted in turn with PS1, then PS2 and finally PS3 antibodies attached to Affi-Gel 10 (10- μ l beads in each case). After washing, antigens were eluted from the antibody support columns with 10 μ l sample buffer (125 mM Tris-HCl pH 6.5, 2% SDS, 10% glycerol), reduced by addition of 2-mercaptoethanol to 5% v/v and components resolved by SDS-PAGE on thin (0.75 mm) 8% gels. The components are all glycoproteins and were visualized by the procedure of Hawkes¹³; nitrocellulose (Western) blots¹⁴ from gels are incubated successively in concanavalin A (Con A) and horseradish peroxidase (HRP), and HRP activity is then detected in a staining reaction. M_s were determined using 29–205K protein markers (Sigma). All procedures have been described previously^{8,9}, as has the isolation of the antibodies. Each PS antibody class contains several independently isolated monoclonal antibodies with identical specificities^{8,9}. *D. melanogaster* embryogenesis lasts for ~22 h at 25°C. The timing of some of the major events is as follows (for review see ref. 12): the cellular blastoderm forms at 2.5–3 h after egg laying, gastrulation begins at 3.5 h, morphologically visible segmentation at ~7 h. Most tissue rudiments become visible between 3 and 10 h and organogenesis continues until shortly before the first-instar larva hatches at 22 h. A moult 24 h later marks the beginning of the second larval instar, which lasts until the second moult at ~72 h after egg laying. The third larval stage ends at 120 h, when the animal pupates and begins metamorphosis.

Fig. 2 PS antigens from larval and adult tissues. Lanes *a–i*, total PS antigens extracted by PS3 antibodies. Subsequent extraction of the lysates with PS1 and PS2 antibodies yielded no further antigens, showing that all complexes contained the common gp110 component recognized by PS3 antibodies. Lanes 1–3 of *l–p* are antigens extracted from lysates by PS1, PS2 and PS3 antibodies in sequence (see Fig. 1 legend). Tissues: *a* and *p*, larval gut; *b*, adult gut; *c*, adult testis; *d* and *m*, larval salivary gland; *e* and *n*, larval brain; *f* and *o*, adult ovary (anterior half); *g*, oocytes; *h*, larval fat body; *i*, larval cuticle (contains epidermis plus attached musculature); *j* and *k*, imaginal disk with and without larval gut lysate, respectively (see below); *l*, larval head (contains most imaginal disks together with larval foregut, brain and some epidermis). In all these antigens, the band in the region of 110K (and often one at ~100K) is recognized by the monoclonal antibody DX4C8 (an antibody that binds to disk antigen gp110 and its 100K breakdown product on Western blots, but not on cells or in solution⁹; data not shown).

Methods. Tissues were dissected out of mature third-instar larvae or young adults of the *D. melanogaster* Barton strain (very similar results were obtained using tissues from Oregon R or Canton S animals), lysed by sonication in non-ionic detergent buffer (see Fig. 1 legend) and antigens extracted by the Affi-Gel 10-bound PS antibodies described above. Equivalent volumes (~50 μ l) of packed tissue were used in each case (this volume required variable numbers of organs: 75 pairs of larval salivary glands and 40 adult testes, for example). Antigen components were resolved by SDS-PAGE, then (with the exception of lanes *j* and *k*) transferred to nitrocellulose and visualized with Con A/HRP (see Fig. 1 legend). *j*, *k*, PS3 antigens were extracted from a lysate of ³⁵S-methionine-labelled imaginal disks¹⁵ after incubation for 2 h at 24°C either alone (*j*) or with the lysate from 50 μ l larval gut tissue (*k*) (in another experiment a similar antigen pattern was obtained after incubation with intact extirpated larval gut). ³⁵S-labelled products were visualized by fluorography¹⁶.



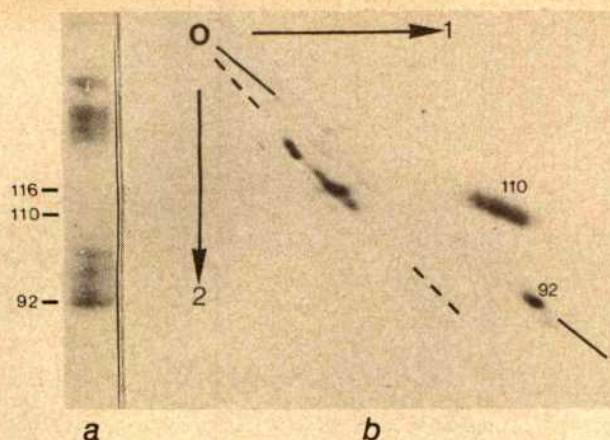


Fig. 3 Two-dimensional non-reducing/reducing SDS-PAGE of late embryo PS antigens. Components of total PS antigen extracted from a lysate of 18-h embryos were first fractionated by non-reducing SDS-PAGE on a thin (0.75 mm) 8% gel (*a*). Note that all PS components have moved away from the 110–120K region, some to higher, others to lower apparent M_r s. A lane identical to that shown in *a* was cut out of the gel, treated for 1 h at room temperature with sample buffer containing 5% 2-mercaptoethanol, then placed on top of a second, slightly thicker (0.9 mm) 8% gel and the reduced proteins re-electrophoresed (*b*). (For details of this procedure see ref. 9.) Products were transferred to nitrocellulose and visualized by Con A/HRP staining. Gp110, which migrates more rapidly in the non-reducing dimension, lies above the diagonal (solid line) of the gel; three components can be resolved in this region (all of which are recognized by antibody DX4C8). Several components of higher M_r were retarded similarly in the first dimension (by ~15–20K) and so, in the two-dimensional gel, all lie below the diagonal on a line that also extrapolates to the origin (broken lines). This behaviour is reminiscent of that of gp116, 120 and 125 of the disk antigens, which contain similar polypeptides⁹. The gp92 doublet glycoproteins, which run identically in each dimension, lie on the diagonal.

might also be regionalized, perhaps localized to entire organs and tissues.

To investigate this possibility, we analysed antigens extracted by PS antibodies from lysates of several larval and adult tissues. Figure 2 shows the SDS gel patterns of total PS antigens (precipitated by PS3 antibodies). As expected, the complexes precipitated by the same antibodies from different tissues differ in their composition, with only the common gp110 occurring in all the antigens. These differences were highlighted in experiments in which tissue lysates were sequentially extracted with PS1, PS2 and PS3 antibodies. For example, larval brain (Fig. 2*n*, lanes 1–3) exhibits only low levels of an antigen that is almost exclusively PS2; in particular, little or no PS1 antigen is detectable, in agreement with immunofluorescence staining results¹¹. In the adult ovary (Fig. 2*o*, lanes 1–3), the population of antigens is composed almost entirely of the PS1 and PS2 subsets, while in the larval gut (Fig. 2*p*, lanes 1–3) complexes recognized only by PS3 antibodies predominate.

It is critical to exclude the possibility that this heterogeneity is an artefact; the reproducibility of the patterns is strong evidence against this. Furthermore, qualitatively identical patterns were obtained with tissues from a number of different fly strains (data not shown). Protease inhibitors were included in all preparations to minimize any effect of a differential occurrence of proteases in different tissues and several experiments were carried out to rule out this possible cause of artefacts. Gut PS antigens contain mainly components of low M_r , which might be candidates for degradation products, and the gut is most likely to contain proteolytic enzymes. Therefore, we incubated ³⁵S-methionine-labelled imaginal disk lysates with gut extracts or intact extirpated gut, before extraction with PS3 antibody. The ³⁵S-labelled PS3 antigen from this preparation was identical to that extracted from control lysates (Fig. 2*j*, *k*). Thus, we

could find no evidence of proteolytic or indeed any other modifying activity and conclude that the differences in the SDS gel patterns between different tissues represent differences in the composition of the complexes. In summary, it seems highly likely that the complex embryonic patterns we have described represent the sum of the different complexes found on the various organs and tissues present.

How are the PS antigens on the various organs, tissues and regions of the organism related? The main characteristics of the antigens, their ubiquity and heterogeneity, are reflected in the form of the complexes: each consists of a characteristic set of variable components together with one component, gp110, common to all of them. The fact that complexes of such different composition, for example, those from salivary gland and adult ovary (lanes 2 of *m*, *o* in Fig. 2), are precipitated by the same antibody, shows that at least some of the variable components are related. All these components (except gp92) migrate abnormally on two-dimensional non-reducing/reducing gels; they all appear on a single line extrapolating back to the origin but running below the gel diagonal. For example, in the case of the total PS antigens from 18-h embryos, six components ranging from 112K to ~140K lie on this line below the diagonal (Fig. 3*b*). The high- M_r components (>110K) of antigens isolated at other stages of development behave similarly (data not shown). Interestingly, the variable components of the disk antigens (gp116–125) show the same anomalous behaviour⁹, and partial proteolysis studies have indicated that these components contain similar polypeptides⁹ (while gp92 and gp110 are not related either to each other or to the other components). It seems likely, therefore, that at least some of the variable components of the embryonic and larval antigen complexes are related both to the variable components of the disk complexes and to one another. These variable components may be products of related genes or of one or a small number of common precursors (transcripts or polypeptides), which are modified according to position and time to give rise to the different tissue-specific components. Preliminary deglycosylation studies suggest that the variations do not stem from differential *N*-glycosylation (M.L. & M.W., unpublished observations).

The relatively indiscriminate expression of PS antigens on the majority of *Drosophila* tissues, as detected by immunofluorescence staining, turns out therefore to be only a superficial view of a more complicated picture. At the finer level of analysis used here, temporal and spatial differences in the composition and distribution of the PS antigens become apparent, and we begin to resolve the heterogeneity of the antigens and their specificity for organs and tissues. In effect, the network of position-specific antigens provides different tissues and regions of the organism with characteristic signatures distinguishing them from their surrounding tissues. It remains to be shown whether and how these signatures are used in the organization of the developing fly; the notion that they are in fact used rests largely on the correlation of the temporal and spatial changes of antigen expression with defined morphogenetic events. The restricted distributions of the antigens on imaginal disks and the changes in expression during development have been discussed previously^{7,8,10,11}. We now know also that the first appearance of the antigens during embryogenesis correlates closely with the onset of morphogenetic processes at gastrulation and that PS-antigen complexity increases dramatically during the ensuing hours when the regional organization of the embryo and the subsequent organogenesis are occurring. Our contention that this complexity results from the summing of the components of different tissue- and region-specific complexes first expressed in these early stages, is supported by the tissue-, organ- and region-specific antigen distributions found later in development.

While a role for the PS antigens in specific cell recognition is attractive, the known properties of the molecules may well be consistent with several other possible functions. The use of monoclonal antibodies specific for isolated variable components should help to elucidate the relationships between the antigens, and the location of their genes and subsequent exploitation of

Drosophila genetics should lead to the isolation of mutants that will allow us to establish the function of the antigens.

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Specific targeting of cytotoxic T cells by anti-T3 linked to anti-target cell antibody

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The specificity of cytotoxic T lymphocytes (T_c) cells is conferred by an antigen-specific receptor, T_i , which in humans is physically associated with an invariant cell-surface glycoprotein, T3 (refs. 1-3). Monoclonal antibodies specific for either T3 and T_i are able to elicit a variety of T-cell responses such as lymphokine production, mitogenesis and cytotoxicity¹⁻¹¹. For example, human T_c cells lyse anti-T3-expressing hybridoma cells, but not cells of other specificity, presumably because anti-T3 on the hybridoma cells binds to T3 on the T_c cells and triggers lysis¹². Here, we have adapted approaches used in a different cytotoxic effector system, antibody-dependent cellular cytotoxicity (ADCC), to alter the specificity of T_c cell. Studies of ADCC¹³ showed that heteroaggregates containing anti-Fc receptor (Fc γ R) antibody cross-linked to a second antibody bind to Fc γ R on ADCC effectors and cause them to kill target cells bearing antigen recognized by the second antibody. The present studies use anti-T3-containing heteroaggregates to re-target human T_c cells to cells for which we have appropriate antibodies, including xenogeneic tumour cells and chicken erythrocytes. These results extend previous observations on the role of T3 in triggering cytotoxicity and suggest that effector cell re-targeting could be used for *in vivo* treatment of neoplasms and other pathogens that express distinctive surface antigens.

Four different human anti-HLA-DPw2 (SB2) cytotoxic T-cell clones, 8.2, 8.4, 8.5 and 8.9 (ref. 14), were used in this study. All four express the T3 and T4 antigens and target-cell lysis mediated by three of the clones (8.4 being the exception) is inhibited by anti-T3 antibody¹⁴. Clones 8.9 and 8.5 were negative for Fc γ R (as determined by flow cytometry¹⁵) and therefore cannot mediate ADCC (clones 8.2 and 8.4 were not tested).

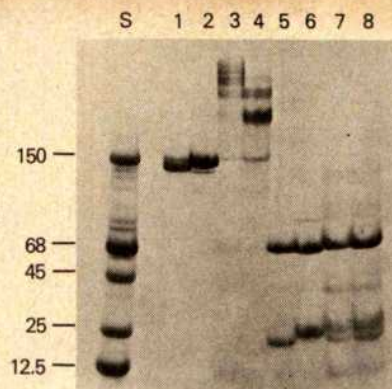


Fig. 1 SDS-polyacrylamide gel electrophoresis of cross-linked antibodies. Lanes 1-4, non-reduced; lanes 5-8, reduced. Lanes 1, 5, OKT3 (anti-T3); lanes 2, 6, 36-7-5 (anti- K^k); lanes 3, 7, OKT3 cross-linked to 36-7-5 (anti-T3 $\times$ anti- K^k), heavier fraction containing mainly trimers and larger oligomers; lanes 4, 8, lighter fraction of cross-linked material, containing mainly dimers and trimers. The cross-linked material reduces to light and heavy chains because a reducible cross-linking reagent was used in their preparation. Note that the cross-linked preparations contain light chains from both antibodies, as expected. Lane S, standards: IgG (relative molecular mass (M_r) 150,000), bovine serum albumin (68,000), ovalbumin (45,000), chymotrypsinogen (25,000) and cytochrome c (12,500).

Methods. OKT3 (Ortho) and 36-7-5²⁰ antibodies were purified from ascites by ion-exchange chromatography and gel filtration²¹. Each protein (5 mg) was incubated with a threefold molar excess of N-succinimidyl-3-(2-pyridyldithiol) propionate (Pharmacia). OKT3 was then reduced and coupled to 36-7-5 as described previously¹³. The hetero-cross-linked antibodies were eluted on a 1.6 $\times$ 90-cm Ultrogel AcA 22 (LKB) column, fractions taken at relative elution volume $K_d = 0.08-0.33$ (heavy fraction) and 0.38-0.54 (lighter fraction). Monomeric IgG elutes at (K_d) 0.63-0.71. Samples were concentrated with a CX10 immiscible membrane (Millipore), and aliquots were denatured and run on a 2-16% gradient slab gel (Pharmacia). Other antibodies used were affinity-purified rabbit anti-DNP antibodies²² and anti-T4 monoclonal antibody^{1,3} (Ortho) purified as described above. In cross-linking anti-T3 to anti-DNP, an eightfold molar excess of SPDP to each antibody was used. This gave larger heteroaggregates than the threefold excess used in the other combinations, but also partially inactivated the antibodies, as shown by the inability of anti-T3 $\times$ anti-DNP to inhibit lysis of M16 cells by clone 8.9 (Table 2).

Cross-linked antibodies were prepared as described in Fig. 1 legend. Two cross-linked fractions were separated from monomeric IgG by gel filtration (Fig. 1). Preliminary experiments showed that both were equally active and they were therefore used interchangeably. In the present study, hetero-cross-linked antibodies are designated 'antibody 1 $\times$ antibody 2', for example, anti-T3 $\times$ anti- K^k .

The four clones were tested for their ability to lyse M16, a human lymphoblastoid cell line bearing the HLA-DPw2 antigen, RDM4, an H-2^k mouse tumour line, and EL-4, an H-2^b mouse tumour line (Table 1). As expected, these clones lyse the M16 target but not the murine tumour cells. Moreover, lysis of M16 by clones 8.9, 8.2 and 8.5 was blocked by anti-T3 $\times$ anti- K^k , showing that anti-T3 remained active in the heteroaggregate. The main finding of Table 1 is that anti-T3 $\times$ anti- K^k causes each of the T_c -cell clones to lyse the K^k -positive RDM4 targets, but not the K^b -expressing EL-4 cells. RDM4 was also lysed if either effector or target cells were preincubated with the heteroaggregates and washed before the experiment (data not shown).

These results suggest that anti-T3 $\times$ anti- K^k cross-links K^k on RDM4 to the T3 portion of the T-cell receptor complex on the T_c -cell clones and triggers the T_c cells to lyse the RDM4 targets. Figure 2 confirms specific intercellular cross-linking, as reflected in the formation of multicellular conjugates between effectors and targets. Figure 2a, b shows that in the absence of the

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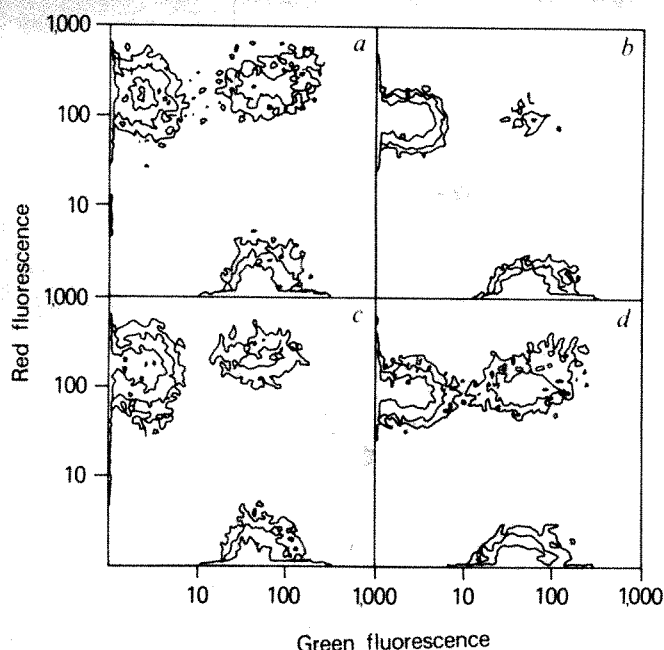


Fig. 2 Conjugate formation between T_c -cell clone 8.9 and tumour lines M16 (a, c) and RDM4 (b, d) in the absence (a, b) or presence (c, d) of anti-T3 $\times$ anti-K^k. Clone 8.9 cells were labelled with fluorescein isothiocyanate as described elsewhere²³. M16 and RDM4 cells (10^7 per ml) were incubated for 10 min at 37 °C with $10 \mu\text{g ml}^{-1}$ N-hydroxysuccinimidobiotin (Sigma) in PBS, pH 8.0, washed and incubated with phycoerythrin-avidin ($20 \mu\text{l}$ of an appropriate dilution of R-phycoerythrin-avidin was added to 10^7 cells in 100 μl of PBS, pH 7.2, and incubated for 30 min at 24 °C). Conjugates were formed by mixing 8.9 cells with M16 or RDM4 cells at a 1:1 ratio, centrifuging and incubating for 20 min at 24 °C. In c and d, effectors were preincubated for 30 min at 0 °C with $10 \mu\text{g ml}^{-1}$ anti-T3 $\times$ anti-K^k before adding the target cells. After the 20-min incubation, cells were gently resuspended with a Pasteur pipette in 1 ml PBS at 0 °C and analysed with a Becton-Dickinson FACS analyser. Free 8.9 cells appear green only, free target cells appear red only and conjugates appear both red and green. 2×10^4 particles were analysed in each sample and contours were drawn through points containing 10, 20 and 40 particles. The percentages of effectors in conjugates were: a, 28%; b, 8%; c, 25%; d, 31%.

heteroaggregate, clone 8.9 forms the expected large number of conjugates with M16, but few conjugates with RDM4. By contrast, in the presence of heteroaggregates, the number of RDM4-8.9 conjugates increases dramatically while the number of M16-8.9 conjugates remains relatively unaffected. The latter observation is consistent with previous studies^{16,17} showing that anti-T3 blocks T_c -cell mediated lysis at a step following conjugate formation.

To test the specificities required for cross-linked antibodies to mediate lysis, we coupled anti-T3 to anti-dinitrophenyl(DNP), anti-T4 to anti-K^k and anti-DNP to anti-K^k, using procedures similar to those described for Fig. 1. Table 2 shows that the anti-T3 $\times$ anti-DNP confers anti-DNP specificity to T_c -cell clones; in the presence of this heteroaggregate, T_c cells lyse TNP-RDM4 and TNP-EL4, but not unmodified EL4 or RDM4. Moreover, DNP hapten in solution strongly inhibits lysis of the haptenated targets. Interestingly, T_c cells will even lyse hapten-modified chicken red blood cells (CRBC) in the presence of anti-T3 $\times$ anti-DNP. Thus, the antibody heteroaggregates seem to override the normal requirement for lysis that T_c -cell receptors bind to molecules of the major histocompatibility complex (MHC) on target cells, as it is unlikely that either CRBC or the murine T_c -cell tumour lines EL4 and RDM4 express MHC molecules, which could be recognized by the class II-specific T_c -cell clones used here.

We next investigated whether anti-T3 must be a component of the antibody heteroaggregate, or whether monoclonal antibodies against any effector cell-surface molecule would trigger lysis. For this, we tested the effect of anti-T4 $\times$ anti-K^k on lysis

Table 1 Anti-T3 $\times$ anti-K^k heteroaggregates re-target T_c cells to K^k-expressing cells

Clone	Anti-T3 $\times$ anti-K ^k	% Specific lysis of target cell		
		M16	RDM4	EL-4
8.9	—	47.0	6.6	0.8
8.9	+	11.5	53.3	0.2
8.2	—	44.9	9.1	3.3
8.2	+	20.4	43.9	—0.7
8.4	—	49.8	7.2	4.3
8.4	+	54.1	32.9	0.8
8.5	—	35.7	1.5	0.6
8.5	+	8.0	30.0	0.4

Lysis was measured in the presence or absence of $5 \mu\text{g ml}^{-1}$ anti-T3 $\times$ anti-K^k at a 10:1 effector: target ratio in a 3-h ^{51}Cr -release assay. Antibody was preincubated with effectors and targets for 30 min at 0 °C, before warming to 37 °C. Human T_c -cell clones 8.2, 8.4, 8.5 and 8.9 are specific for the HLA-DPw2 antigen^{14,18}. M16 (ref. 19) is a human Epstein-Barr virus-transformed lymphoblastoid cell line that expresses HLA-DPw1 and -DPw2 antigens. RDM4 and EL4 are H-2^k and H-2^b murine T_c -cell tumour lines. Per cent lysis was calculated as described elsewhere¹³, and spontaneous release was 8.9, 5.2 and 6.8% for M16, RDM4 and EL-4, respectively.

of M16 and RDM4. Results from experiments similar to those shown in Fig. 2 confirmed that anti-T4 $\times$ anti-K^k caused conjugates to form between clone 8.9 and RDM4 (21% of effectors were conjugated in the presence of heteroaggregate, 9% in its absence). However, this cross-linked preparation neither promoted lysis of RDM4 nor significantly blocked lysis of M16 by clone 8.9 (it has previously been demonstrated¹⁸ that anti-T4 does not inhibit lysis mediated by clone 8.9) (Table 2). In a second experiment (not shown in Table 2), T_c -cell clone 8.9 was treated with 1.5 mM trinitrobenzene sulphate and tested for lysis against RDM4 in the presence of anti-DNP $\times$ anti-K^k. Control experiments showed that anti-DNP $\times$ anti-K^k promoted specific conjugate formation between TNP-8.9 and RDM4 and that the TNP-8.9 cells were able to lyse M16. However, TNP-8.9 cells did not lyse RDM4 cells in the presence of anti-DNP $\times$ anti-K^k (<2% lysis over a concentration range of 5–40 $\mu\text{g ml}^{-1}$ heteroaggregate). Therefore, cross-linking of target cells specifically to the T4 molecule and to a presumably large number of TNP-modified components on the effector cells did not lead to lysis. These data suggest that the cross-linking of target cells directly to the T_c -cell receptor complex is a requirement for lysis. In support of this view, earlier experiments¹² showed that several hybridoma cells expressing antibodies against effector cell-surface components other than T3 are not lysed by T_c -cell clones. In addition, Kranz *et al.*¹¹ have recently shown that a mouse T_c -cell clone will lyse targets to which anti-Ti antibodies were attached, but not targets bearing antibodies that bind to other effector cell-surface components.

From current results, there seems to be a striking parallel in the way in which ADCC effector cells and T_c cells lyse target cells; in both cases, direct attachment of the target cells to the appropriate receptor (Fc γ R on ADCC¹³ effectors or T_c -cell receptor complex on T_c cells) leads to target cell lysis, and attachment to other cell-surface components on the effector cell does not. These data strongly suggest that the target-specific receptors in both systems not only mediate conjugate formation, but also have an essential role in the post-conjugate phase of the lytic process. T3 is one cell-surface component that is clearly involved in the post-conjugate phase of T_c -cell mediated lysis, because anti-T3 blocks lysis but does not inhibit conjugate formation. Nevertheless, binding of the target cell directly to T3 results in target cell death. This suggests that the local aggregation of T3 at the cell/cell interface may be instrumental in triggering lysis, and that soluble anti-T3 blocks normal T_c -cell lysis by preventing local T3 aggregation.

We have demonstrated here that the specificities of T_c cells can be altered *in vitro* simply by treating them with antibody heteroaggregates. In fact, we have now been able to direct T_c

Table 2 Specificity of T_c -cell lysis in the presence of hetero-cross-linked antibodies

Target	None	Anti-T3 $\times$ anti-K ^k	% Specific lysis of target cells by clone 8.9			
			Antibody added			
			Anti-T3 $\times$ anti-K ^k + anti-T3	Anti-T3 $\times$ anti-DNP	Anti-T3 $\times$ anti-DNP + DNP	Anti-T4 $\times$ anti-K ^k
M16	37.8	-7.0	-29.7	22.3	—	29.9
RDM4	-8.7	38.2	2.0	-8.6	—	2.1
EL-4	-4.9	-4.2	—	-4.3	—	—
CRBC	1.1	—	—	1.3	0	—
M16-TNP	55.1	—	—	32.3	43.8	—
RDM4-TNP	-8.1	—	—	40.5	7.4	—
EL-4-TNP	0.4	—	—	49.1	0.3	—
CRBC-TNP	0.8	—	—	63.9	0	—

Experiments were performed as described in Table 1 legend using clone 8.9 cells as effectors. Clone 8.5 also lysed TNP-CRBC in the presence of anti-T3 $\times$ anti-DNP in a hapten-specific manner (data not shown). In all experiments shown, $10 \mu\text{g ml}^{-1}$ cross-linked antibody was used, but 20 and $40 \mu\text{g ml}^{-1}$ of anti-T4 $\times$ anti-K^k were also tested, with results similar to those shown here. Anti-T3 ($50 \mu\text{g ml}^{-1}$, column 4) and DNP- ϵ -aminocaproate (0.1 mM , column 6) were used as inhibitors. DNP- ϵ -aminocaproate did not inhibit lysis of M16 by clone 8.9 (data not shown). Target cells were modified with TNP by incubating them for 10 min at 37°C in phosphate-buffered saline (PBS), pH 7.4, containing 3 mM trinitrobenzene sulphonate. (—) Indicates experiment not done. Negative values for per cent lysis indicate that chromium release was less than spontaneous release. In the test sample, spontaneous release values for the various targets were: M16, 28.6%; RDM4, 11.4%; EL-4, 12.4%; CRBC, 2.9%; M16-TNP, 7.4%; RDM4-TNP, 15.4%; EL-4-TNP, 4.5%; and CRBC-TNP, 3.0%.

cells to lyse every target cell for which we have had an appropriate anti-target cell antibody. Based on these observations, it is reasonable to propose that T_c cells could be directed against various pathogenic cells *in vivo*, for example against tumour cells. By using anti-T3-containing heteroaggregates, presumably all T_c cells, and not a limited number of clonotypes, would be targeted to the tumour. In addition, such heteroaggregates could also target T-helper cells to a tumour (or other lesion) where they could serve as a source of lymphokines. Finally, appropriate heteroaggregates could be used to target both T cells (using anti-T3-containing heteroaggregates) and macrophages, granulocytes and natural killer cells (using anti-Fc γ R heteroaggregates) to the same lesion. Such strategies might greatly augment the normal immune response against tumours and other pathogenic cells, and could also provide insight into the normal role of various effector cells in combating infections and neoplasms.

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Note added in proof: Staerz *et al.*²⁴ have recently shown that anti-Ti-containing heteroaggregates will re-target murine T_c cells.

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Pre-B cells in κ -transgenic mice

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Recent experiments have shown that the microinjected κ -chain gene of transgenic mice is expressed in a tissue-specific fashion only in B lymphocytes^{1,2}. The next step was to determine whether, within the B-lymphocyte lineage, the κ -chain gene was expressed in a normal developmental fashion. Normally, only μ heavy(H)-chain genes, and not κ -chain genes, are expressed in pre-B cells³. To obtain cloned cell lines derived from early cells of the B-cell lineage, we transformed bone marrow cells from κ -transgenic mice with Abelson murine leukaemia virus (A-MuLV) and tested the resultant cell lines for the retention of the κ transgene and its expression in RNA and protein. We found that cells with the pre-B phenotype exist in κ -transgenic mice. We further observed that in A-MuLV-transformed cell lines from a κ -transgenic mouse with a high copy number of the transgene, the proportion of cell lines expressing κ (transgenic κ) was higher than in cell lines from normal or low copy number transgenic mice.

Bone marrow was obtained from two 4-week-old transgenic mice and one normal sibling from the same litter. The parents were a male transgenic mouse (no. 48, ref. 2) and a normal female C57BL mouse. A portion of the bone marrow cells was directly transformed by A-MuLV⁴; the rest was first cultured for 3-4 months *in vitro*⁵ and then transformed with A-MuLV.

Kidney DNA from the three donor mice was tested for the presence of the transgene by dot hybridization with a constant region (C_κ) probe and pBR322 as described previously¹. One donor had not inherited the transgene (normal littermate), whereas the two transgenic donors had different copy numbers of the transgene (see below). Apparently the father, no. 48 (ref. 2), contained mosaic germ cells, whereas only the high copy transgenes were observed in the tail DNA (R. L. O'Brien and U.S., unpublished).

All the A-MuLV-transformed cell lines from normal and transgenic mice produce μ -chain RNA (Fig. 1, Table 1). The levels of μ RNA are variable: some cells produce very low

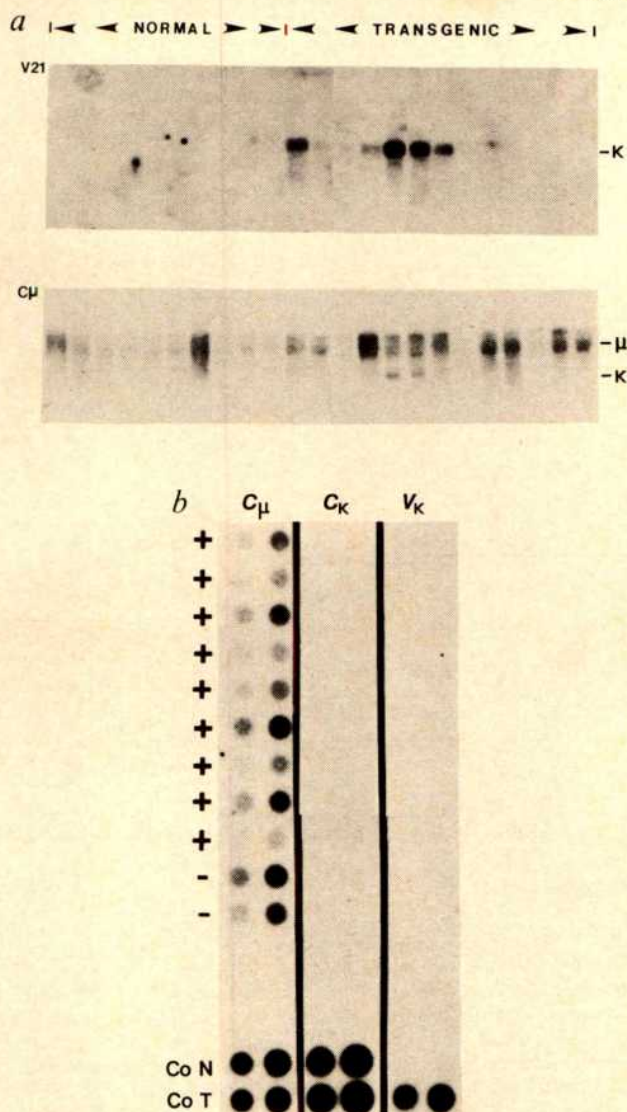


Fig. 1 RNA analysis. *a*, Northern blot of RNA from A-MuLV-transformed cell lines obtained directly from fresh bone marrow (see text). Each lane contains 10 μ g total cellular RNA. The first 10 lanes are from cell lines of normal littermates, the next 7 are from transgenic cell lines of the high copy number mouse, the last 6 are from the low copy number mouse. Top panel shows hybridization with a M.13 DNA probe containing the V_{κ} region of MOPC-21 (the transgene)¹. Bottom panel shows the same blot, first hybridized with V_{κ} M.21 and then rehybridized with a C_{μ} -containing probe⁹. Symbols μ , κ , positions of normal μ and κ mRNAs. *b*, Dot hybridization of RNA from A-MuLV-transformed cell lines. These clones were derived by A-MuLV transformation of bone marrow cells cultured for 3–4 months *in vitro* (see text). The dots were hybridized with the probes indicated: C_{μ} , C_{κ} and V_{κ} M.21; RNAs from transgenic mice (+) and normal littermates (–). CoN, control RNA from normal spleen; CoT, control RNA from transgenic spleen. Each RNA sample was dotted in two dilutions (1 and 3 μ g for C_{μ} and C_{κ} ; 2 and 6 μ g for V_{κ}).

amounts, which may in some cases be the reason for the lack of detectable μ protein (see below). The μ RNA size appears normal, probably representing the secreted and membrane forms of the protein. The cell lines also have rearranged H-chain genes on both chromosomes, indicated by hybridization with a joining segment (J_H) probe (see Fig. 2*a, b* for the long-term bone marrow culture-derived cell lines; data not shown for the direct transformants). Thus, the presence of the κ transgene does not prevent the rearrangement or expression of endogenous μ -chain genes, nor does it seem to impose the rearrangement of particular H-chain genes; among the different A-MuLV-transformed cell lines we found several different patterns of J_H -gene rearrange-

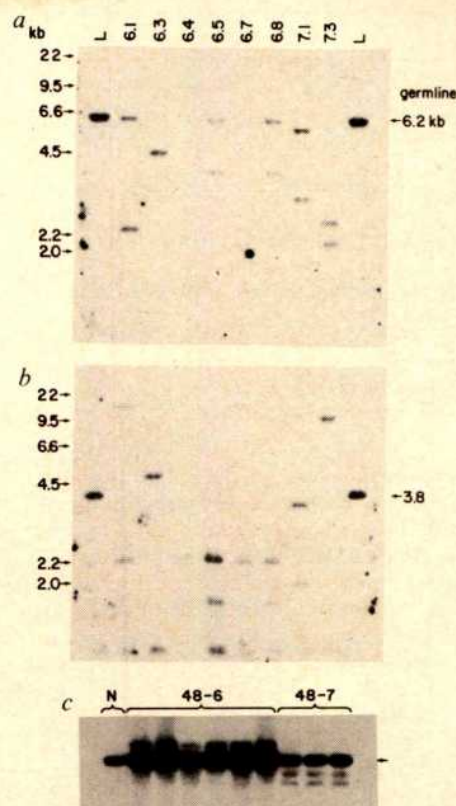


Fig. 2 Southern blots of DNAs of A-MuLV-transformed cell lines. DNAs were digested with *Eco*RI (*a*) or *Xba*I (*b*) and Southern blots were prepared as described previously¹⁰ and probed with nick-translated DNA corresponding to the J_{H2} , J_{H3} and J_{H4} regions¹⁰. DNAs were from cell lines transformed by A-MuLV after prolonged culture of the bone marrow cells (see text). L, liver; 6.1–6.8, cell lines derived from mouse 48-6; 7.1 and 7.3, from mouse 48-7. It seems that 6.1 = 6.7 and 6.4 = 6.5 = 6.8. DNAs from direct A-MuLV transformants were digested with *Eco*RI and probed with a C_{κ} probe cloned in M13 (ref. 1). *c*, Cell lines are from normal littermates (N) and from transgenic mice 48-6 and 48-7. Arrow, tandemly repeated multiple copies of the κ transgene (14.8 kb) (~19 copies in 48-6, 5 in 48-7 cell lines) and germ line C_{κ} genes (15.2 kb).

ment. This provides further evidence that most lines are probably derived from transformation of different, independent cells, but there may be some duplication in the cell clones (Fig. 2*a, b*). Most of the cell lines produce μ protein (Fig. 3, Table 1), indicating that the μ RNA is functional. These results are analogous to previous findings with more mature B cells from transgenic mice that were immortalized by fusion with a hybridoma cell⁶, making it unlikely that the results described here are an artefact of the method of immortalization.

The differential copy number of the κ transgene was retained in the direct A-MuLV-transformed cell lines from the two mice (Fig. 2*c*). This Southern blot also shows additional bands, probably integration fragments, which differ in the cell lines from mice 48-6 and 48-7, suggesting that the transgenes are probably integrated at different sites. With respect to κ RNA, the cell lines from the two transgenic mice behaved differently; 71% of cells from the low copy number mouse 48-7, like the cells from the normal mouse, did not produce κ RNA despite the presence of κ transgenes (Table 1). The proportion of κ -producing cells from mouse 48-7 is within the range normally found in A-MuLV-transformed bone marrow cells³. Thus, even though the κ transgene is expressed in a tissue-specific manner², its presence in a B cell is not sufficient for its expression at a detectable level. True pre-B cells exist in 48-7 κ -transgenic mice. Because μ RNA is produced in these pre-B cells, transcriptional factors for H-chain genes must be present. The lack of transcription from the rearranged κ transgene suggests that different transcriptional

Table 1 Expression of κ - and μ -chain genes in A-MuLV-transformed cells from κ -transgenic mice

Cell donor	No. of cell lines*	C_{κ}	RNA V_{κ} M.21	C_{μ}	Protein†	μ	Pre-B (μ RNA only) total
Direct A-MuLV lines							
Normal littermate	12-	1+	0	11+ 1 (ND)	0/5+	3/5+	11/12 (92)
Transgenic no. 48-6 (high copy)	13+	12+	11+	13+	9/13+	11/13+	1/13 (8)
Transgenic no. 48-7 (low copy)	7+	2+	1+	7+	1/7+	6/7+	5/7 (71)
A-MuLV lines after bone marrow culture							
No. 48-6	7+	7-	7-	7+	0/7+	4/7+	7/7 (100)
No. 48-7	2+	2-	2-	2+	0/2+	1/2+	2/2 (100)

* -, Without; +, with transgene.

† Not all cell lines were tested for protein. ND, not done.

factors are responsible for H-chain and κ transcription and that in pre-B cells only H-chain gene transcriptional factors exist. Alternatively, the κ transgene and normal κ -chain genes may not be in an open chromatin conformation in pre-B cells. Lastly, the differential transcription of μ - and κ -chain genes may be related to differential strength of response to the same transcriptional factors, or cofactors. It has been suggested that the tissue-specific transcriptional 'enhancer' of H-chain genes is ~20 times stronger than the analogous enhancer of κ -chain genes⁷. If the amount of transcriptional factors in pre-B cells is limiting, transcription from μ -chain genes would be favoured over κ -chain genes. The amount of μ RNA in the cell lines is at least six times lower than in spleen (Fig. 1b), which indicates a lower and perhaps limiting level of transcriptional factors.

Limiting amounts of common transcriptional factors for μ - and κ -chain genes could also explain why there was a high frequency of κ RNA-producing cells in the high copy number transgenic cell lines (Fig. 1a, Table 1). Most of the direct A-MuLV cell lines from mouse 48-6 contained detectable levels of κ RNA. The cells from mouse 48-6 have about four times as many copies of the transgene as those from mouse 48-7 (Fig. 2c). Even though the κ -transcriptional enhancer may be weaker than that of μ , its presence in ~19 copies in cells of mouse 48-6 may effectively compete with μ for occasional transcription, leading to a detectable level of κ RNA.

It seems unlikely that the difference in κ frequency of cell lines derived from mice 48-6 and 48-7 results from complete transcriptional inactivation of the transgenes in the low copy number mouse. First, we found one cell line from mouse 48-7 which transcribed the κ transgene (Table 1). Second, the levels of κ RNA encoded by the transgene in spleen cells from the two mice was the same (data not shown). Besides differential transcription, differential processing or stability of the messenger RNAs could be responsible for the relative levels of μ and κ RNAs. This would require specific processing factors and/or nucleases for μ and κ RNAs, and for the κ RNAs in the κ -positive and κ -negative A-MuLV B-cell lines. These possibilities need to be explored further, but other evidence indicates an important role for transcriptional control in the regulation of immunoglobulin mRNA levels⁸.

The distribution of κ -producing cells from mouse 48-6 between the cell lines derived by the two methods of transformation was strikingly different. No κ -producing cells were found in the transformants from prolonged bone marrow cultures (Fig. 1b, Table 1). Surprisingly, these cell lines from mouse 48-6 had lost most of the copies of the transgene and only retained a low copy number, similar to the cell lines from mouse 48-7 (not shown). This finding strengthens the argument that in the high copy cell lines the large number of κ -chain genes effectively competes for limiting transcriptional factors (see above), but alternative explanations are under investigation.

In conclusion, the data reported here support the concept of sequential activation of first H-, then κ -chain genes³. The molecular basis for this control, which aids allelic exclusion of κ genes⁶, remains to be determined. To effect allelic exclusion

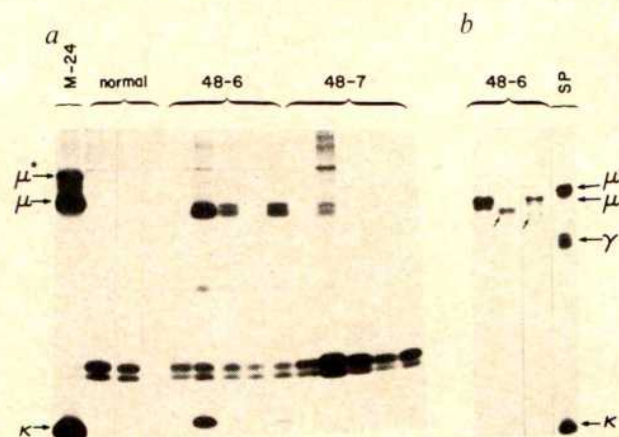


Fig. 3 Analysis of immunoglobulins produced by A-MuLV transformed cell lines. *a*, Direct A-MuLV transformed; *b*, A-MuLV transformed after prolonged bone marrow culture. Cells were grown in tunicamycin and metabolically labelled with ³H-leucine¹⁰. Immunoprecipitation with polyclonal rabbit anti-mouse immunoglobulin and electrophoresis on SDS-polyacrylamide gels was carried out as described previously¹⁰. M-24 is an established A-MuLV transformed B-cell line. Normal, 48-6 and 48-7 are cell lines derived from the three respective mice. SP, normal spleen (not treated with tunicamycin); μ^* and μ , glycosylated and unglycosylated forms of μ , respectively. Oblique arrows indicate lighter forms of μ , which were consistently observed in these two cell lines; presumably they represent translates from incomplete μ RNAs (such as *D-J-C-μ*-encoded). The double bands above the κ -chains are unexplained artefacts seen in some, but not all, immunoprecipitates with the same antiserum.

of κ genes⁶, transcriptional factors for κ -chain genes seem to be required at a high level either before or at the same time as κ -chain gene rearrangement.

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A transposon-like element in human DNA

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Mobile genetic elements have been reported in prokaryotes, plants, yeast and *Drosophila* (reviewed in refs 1, 2). The only transposon-like sequences reported for mammalian organisms are closely related to retroviruses³⁻⁷, although undoubtedly other transposon families exist within the mammalian genome. Although mobile genetic elements can only be identified as such if their mobility can be demonstrated in existing populations, transposon and transposon-like elements share several common biochemical and structural features^{1,2}. Here we demonstrate that a repetitive human sequence has many of the diagnostic features of transposable elements. This 2.3-kilobase (kb) transposon-like element contains two flanking long terminal repeat (LTR)-like 350-base pair (bp) repetitive sequences, each of which begins with the sequence 5' TG... and ends with ...CA 3'. The transposon-like element is bounded by 5-bp direct repeats. Discrete-length polyadenylated transcripts from HeLa cells are homologous to the transposon-like element. Members of this transposon-like family are found in extrachromosomal circular DNA molecules.

Sun *et al.*⁸ previously described the 'O' family of repetitive sequences. A slightly different representation of two members of this family (see below) indicates that the 350-bp O family repeats begin with TG and end with CA (B. Baum, personal communication), a hallmark of the LTRs that flank transposable

elements¹⁻³. Therefore, the O repeats may be solitary LTRs, and additional O family members could be linked to longer transposon-like elements. To test this interpretation, we determined the DNA base sequence of additional members of the O family, which were isolated as described previously⁸.

One such recombinant DNA clone, λ THE 1-A, contains two members of O at opposite ends of a 1.9-kilobase (kb) *Bgl*/II restriction fragment (Fig. 1a). (This structure is called a transposon-like human element, and corresponding clones are designated THE 1; the O family is now called the 'O-LTR' family.) Subcloned restriction fragments of λ THE 1-A (Fig. 1a) were used as hybridization probes for additional λ THE clones as well as genomic DNA fragments. A 2-kb sequence flanked by two O family repeats is also observed in clones λ THE 1-B and -C (Fig. 1a). Because these three recombinant DNAs share homologous sequences and similar maps, they must be members of a repetitive sequence family. The following hybridization experiments confirm the repetitive nature of the THE 1 family.

Human DNA digested with the restriction enzymes listed in Fig. 1a was hybridized to O-LTR repeats and subcloned restriction fragments of λ THE 1-A (Fig. 1a). The *Bgl*III digest of human DNA illustrates the main results of our study (Fig. 1b). The 350-bp O-LTR repeat hybridizes to a highly repetitive 1.9-kb *Bgl*III fragment in addition to restriction fragments of other lengths (Fig. 1b), indicating that O-LTR repeats are associated with longer repetitive sequences. Subclones derived from the longer repetitive element of λ THE 1-A hybridize to many of the same genomic restriction fragments as the O-LTR family, with noticeably lower background (Fig. 1a, b). Comparison of the λ genomic clones THE 1-A, -B and -C reveals restriction site polymorphisms (Fig. 1a), many of which correspond to minor genomic restriction fragments that can be arranged as a consensus restriction map (Fig. 1a). Evidently, most of the sequences that are homologous to THE 1 family repeats share a single consensus sequence.

Dot-blot and library screening hybridizations indicate that there are ~30,000 O-LTR repeats and 10,000 copies of the internal element within human DNA (data not shown). These

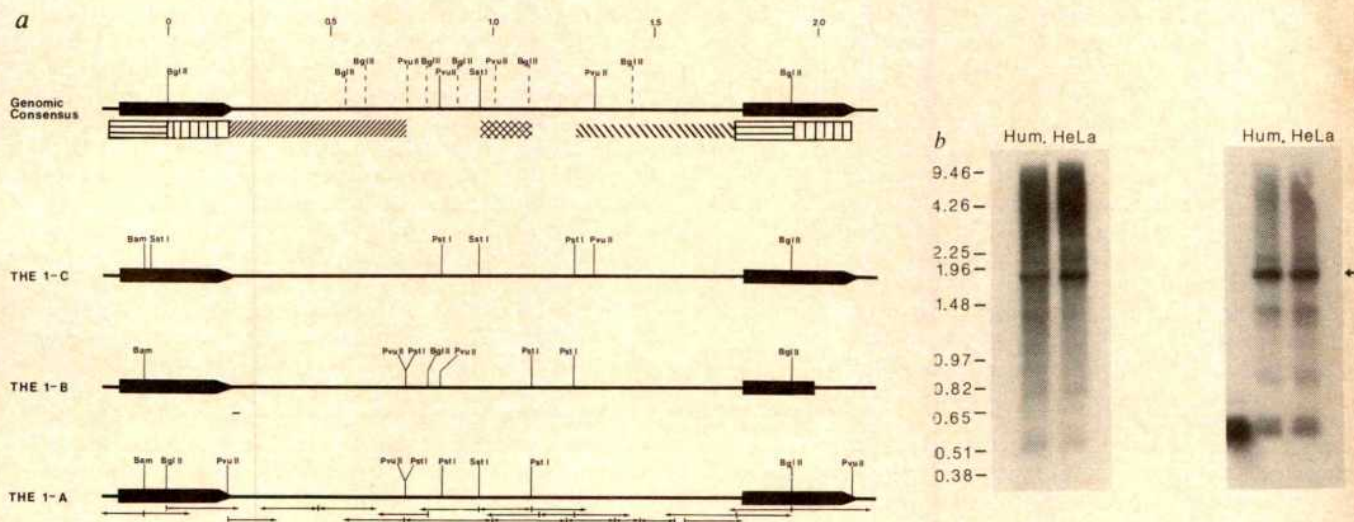


Fig. 1 *a*, Genomic and clone restriction maps. Restriction fragments of THE 1-A (indicated schematically by the cross-hatched and shaded bars under the genomic consensus map) were subcloned and used as hybridization probes to the indicated restriction digests of λ THE 1-A, -B and -C and genomic DNA. Genomic digests are complicated by the appearance of a number of weak and presumably polymorphic restriction fragments (*b*). Relatively rare sites are indicated in the consensus map by dotted lines; strong sites are indicated by solid lines. The right LTR of λ THE 1-B is deleted, a finding which has been confirmed by limited base sequencing. The base sequencing strategy used for λ THE 1-A is indicated by the arrows positioned under the map of λ THE 1-A. These restriction fragments were subcloned into M13 and their sequences were determined by Messing's modifications of the Sanger dideoxynucleotide procedure and limited Gilbert-Maxam sequencing of refractory regions. *b*, Mapping of genomic restriction digests. Placental and cell culture DNAs (labelled human and HeLa respectively) digested with *Bgl*II were hybridized to the LTR family (pBRQ₄ in ref. 8) and pUC19 subclone of the 1.9-kb *Bgl*II fragment of λ THE 1-A as depicted in *a*. A highly repetitive 1.9-kb *Bgl*II fragment, as indicated by the arrow, as well as several minor bands are revealed by this hybridization. The autoradiograph was exposed overnight without an intensifying screen. The minor bands can be rationalized according to the rarer sites in the consensus map of *a*.

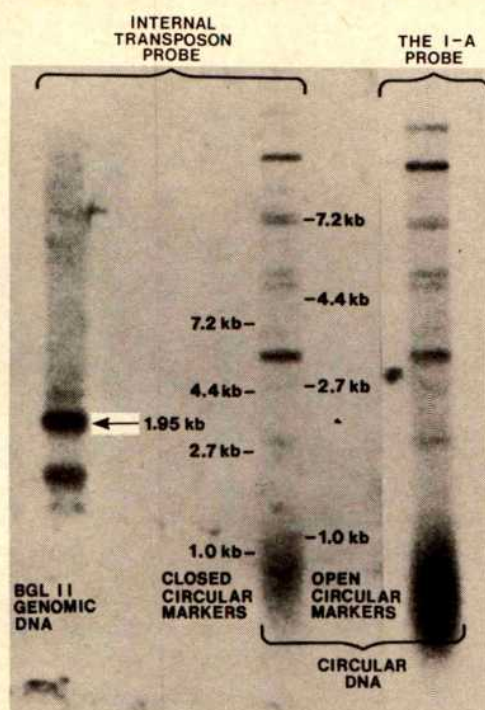


Fig. 4 Covalently closed circular DNAs from the Jurkat lymphoblastoid human cell line were isolated and resolved by gel electrophoresis^{12,13}. Blots of these gels were hybridized to subclones corresponding to an internal 0.38-kb *Pst*I fragment of λ THE 1-B (left panel as indicated), the 1.9-kb *Bgl*II fragment of λ THE 1-A (right panel as indicated) or the LTR-specific hybridization probe (pBR0₄ of ref. 8; data not shown). The right and left panels, which correspond to gels that were run for slightly different times, are juxtapositioned to indicate the coincidence of the bands. Circular DNA marker lengths are indicated in the left panel, which also includes a *Bgl*II digest of genomic DNA revealing the diagnostic 1.9-kb band described in Fig. 1a. As an important negative control, neither pBR nor M13 DNAs exhibited any hybridization to these gels.

that of typical retroviruses. Therefore, THE 1 family repeats should be regarded as a new class of repetitive transposon-like sequences.

Blot hybridization reveals the THE 1 structure in monkey but not in rodent DNA, so that the mobility of these sequences over long evolutionary times seems certain. However, blot hybridization of a sequence flanking THE 1-B to the DNAs of 19 individuals indicates that this particular member of the family is fixed in the human genome (data not shown). The mobility or relative immobility of this family remains to be determined.

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Lymphokine dependence of *in vivo* expression of MHC class II antigens by endothelium

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Changes in the expression of class II antigens of the major histocompatibility complex (MHC) have an integral role in the regulation of immune responses, and are brought about *in vitro* by soluble mediators¹⁻⁴. However, the mechanism that underlies *in vivo* expression of MHC class II antigens in, for example, endothelial cells in the absence of immunological stimulation has not been studied. We demonstrate here that expression of MHC class II antigens is not a constitutive property of endothelial cells, for MHC class II antigen-positive endothelial cells do not express these antigens during treatment with the immunosuppressive agent cyclosporin A. *In vivo* MHC class II antigen expression by canine endothelial cells is therefore dependent on factors, probably the lymphokine γ -interferon produced by the immune system, whose secretion is inhibited by cyclosporin A.

γ -Interferon (IFN- γ) has been identified as the lymphokine responsible for the augmented MHC class II antigen expression observed on monocytes *in vitro*⁴, although recently an intermediate messenger, produced by murine tumour macrophages in response to IFN- γ , has been reported to be involved in this process⁵. The MHC class II antigen-inducing capacity of IFN- γ has also been clearly demonstrated *in vitro* for endothelial cells from human umbilical vein⁶ and fibroblasts⁷. Furthermore, there is evidence that the expression of these antigens changes on immunological stimulation⁸⁻¹². For example, rat skin^{9,10} and gut epithelium¹⁰, both normally MHC class II antigen-negative cells, express these antigens during graft-versus-host disease and local infections¹¹. We therefore investigated whether the normal 'physiological' expression of MHC class II antigens is dependent on factors such as IFN- γ .

An *in vivo* model was selected that assessed the effect of lymphokines on normal endothelial cell MHC class II antigen expression by analysing the response to cyclosporin A; this compound has been shown to inhibit the *in vitro* production of the factor responsible for MHC class II antigen expression¹³⁻¹⁵. The canine model was chosen because the dog resembles humans¹⁶⁻¹⁹ in exhibiting MHC class II antigens on the capillary endothelial cells of the skin and the glomerular and peritubular capillary endothelium of the kidney.

MHC class II antigen expression was studied in skin and kidney biopsies from five dogs treated with cyclosporin A. The drug was given once daily as an oral solution (Sandoz), and drug blood levels were monitored by HPLC and maintained between 0.2 and 0.4 mg ml⁻¹. The cyclosporin A doses administered ranged from 15.0 to 22.5 mg per kg per day. Drug treatment did not affect the health of the dogs. Renal function, as assessed by serum creatinine and urea concentrations, was unaffected; creatinine concentrations were 40-60 μ mol l⁻¹ during treatment (normal range in dogs 30-120 μ mol l⁻¹). Wound healing of the biopsy incisions was also unimpaired.

The expression of MHC class II antigens during cyclosporin A treatment was studied in tissue biopsies by indirect immunofluorescence using two monoclonal anti-MHC class II antibodies (Fig. 1). Renal biopsies taken from the first dog studied on days 10, 19 and 32 of treatment revealed that MHC class II antigens were present before treatment (Fig. 1a), but could not be detected during treatment (Fig. 1b); the antigens were consistently absent from the renal vasculature in all biopsies taken after 10 days of cyclosporin A treatment.

Endothelial cells were identified using the marker protein, FVIII-related antigen²⁰ (Fig. 2). In renal biopsies from untreated dogs, both antibodies stained endothelium of glomerular capil-

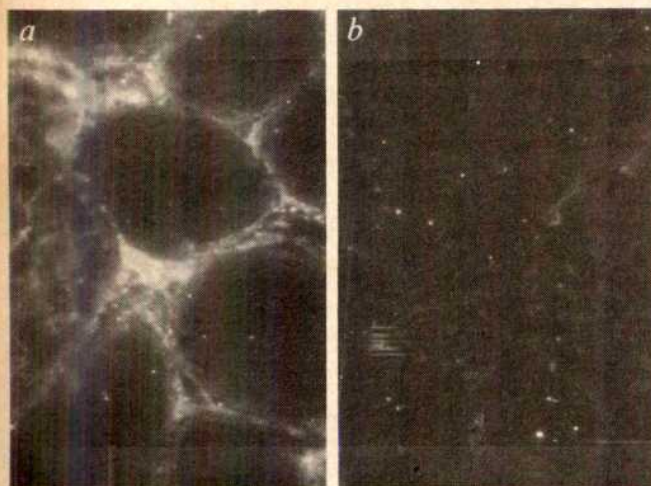


Fig. 1 Distribution of MHC class II antigens in the kidney before (a) and during (b) treatment with cyclosporin A ($\times 400$). Monoclonal antibodies 7.2 (ref. 25) and 7.5.10.1 (ref. 26), known to cross-react with canine MHC class II antigens²⁷, were used in the ascites form. Both monoclonal antibodies gave identical patterns of immunofluorescence. The reactivity of antibody 7.5.10.1 is shown. a, A biopsy taken before treatment, showing part of a glomerulus (left margin) and several unstained tubules; the glomerular endothelium stained for MHC class II antigens, as did peritubular capillary endothelium. Biopsy b, taken after 10 days of cyclosporin A administration, is devoid of antigens reacting with anti-MHC class II reagents, even at antibody concentrations 10-fold higher than the concentration that appeared on titration to be optimal in biopsies of untreated dogs.

Methods. Healthy beagles from the colony of the University of Maastricht were regularly screened for infections; the last vaccination had been given at least 12 months before the experiment. Tissue specimens, obtained by open biopsies, were snap-frozen in isopentane, cooled on dry ice. Tissue sections ($4\ \mu\text{m}$) were fixed in acetone at -20°C and rehydrated in phosphate-buffered saline (PBS) at room temperature. Subsequently, sections were incubated for 30 min at room temperature with the desired antibody, washed for 15 min in PBS and incubated with an appropriate fluorescein-labelled second antibody. Antibodies were used at twice the concentration shown by titration to be still saturating, to achieve maximal sensitivity of the fluorescence technique. After a final wash the section was embedded in glycerol.

laries and vasculature around the tubules, whereas tubular cells and basement membrane structures were not stained. Before and during treatment with cyclosporin A, the distribution of FVIII-related antigen in endothelium was unchanged. However, MHC class II antigen distribution differed before and during treatment. Our findings were confirmed in a second dog treated with cyclosporin A and biopsied on days 10, 20 and 33 of treatment. In three other dogs, MHC class II antigens could not be detected in renal biopsies taken on day 20 of treatment, whereas the distribution of FVIII-related antigen remained unchanged.

Table 1 summarizes the results of skin and kidney biopsies. The expression of FVIII-related antigen was similar before and during treatment. However, endothelial expression of MHC class II antigens was absent in biopsies taken on day 10 of treatment, whereas before treatment the antigens were clearly present on endothelial cells. Continued treatment until day 25 resulted in a continued absence of the antigens on endothelial cells, whereas the distribution of FVIII-related antigen was unaffected. The fact that the distribution of this marker antigen was unchanged with treatment makes it unlikely that the disappearance of MHC class II antigens from the vasculature of both skin and kidney was caused by a toxic effect of cyclosporin A on endothelium.

In contrast to the disappearance of the endothelial MHC class II antigens, the expression of these antigens by a second vascular bed, the Langerhans cells of the skin, was unaffected by cyclo-

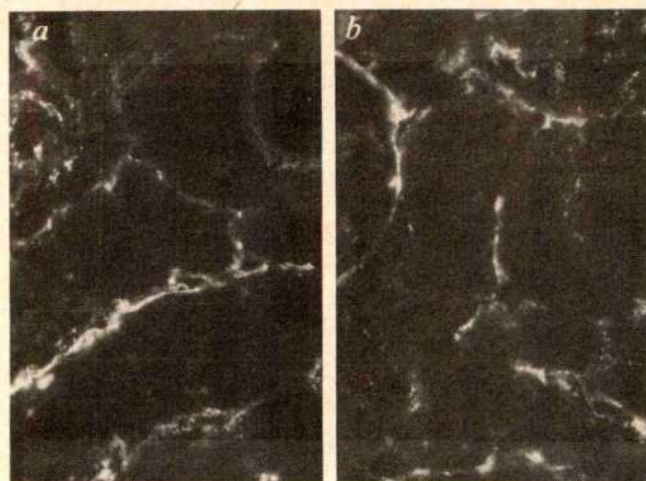


Fig. 2 Distribution of FVIII-related antigen in the kidney before (a) and during (b) treatment with cyclosporin A ($\times 400$). Antiserum specific for dog FVIII-related antigen²⁸ was used to identify endothelium in tissue sections. In biopsies a and b, taken, respectively, before and after 10 days' cyclosporin A administration, endothelial cells of glomerular and peritubular capillaries are stained. In a, a glomerulus is partially visible in the upper left-hand corner; b, glomeruli are visible in the upper right-hand corner and on the left margin of the photomicrograph.

sporin A treatment (Table 1). Whereas treatment for 10 days resulted in the loss of antigens from endothelium, treatment for as long as 25 days caused no change in MHC class II antigen expression on skin Langerhans cells.

In vitro MHC class II antigen expression on endothelial cells is induced and maintained by lymphokines. Cyclosporin A does not influence lymphokine-induced MHC class II antigen expression on endothelial cells *in vitro*, but has an effect via inhibition of the production of lymphokines responsible for antigen expression^{13,14}. Similarly, the expression of MHC class II antigens on endothelium *in vivo* seems to be lymphokine-dependent, as treatment with cyclosporin A, which inhibits the production of the relevant factors, results in the observed decreased expression of MHC class II antigens on endothelium in skin and kidney. Previous studies have already suggested that the immune system affects the expression of these antigens⁸⁻¹². The present study now shows that the immune system sustains the normal expression of MHC class II antigens on endothelium by means of lymphokines.

Although the nature of the lymphokine(s) involved *in vivo* is unknown, it seems likely to be IFN- γ , because this lymphokine is known to induce the expression *in vitro* of MHC class II antigens^{4,6,7} and its production is inhibited by cyclosporin A¹⁵. Our data strongly suggest that basal secretion of IFN- γ ²¹ is the cause of the 'normal' MHC class II antigen expression on endothelial cells. The expression of MHC class II antigens *in vivo* on capillary and venous endothelium and not on arterial endothelium suggests a difference in the sensitivity of these cells to IFN- γ , since, *in vitro*, antigen expression by arterial endothelium can be induced by lymphokines^{13,14}. During immunological stimulation, high concentrations of IFN- γ can induce the expression of MHC class II antigens on cells with low sensitivity to this lymphokine. Differences in reactivity to lymphokines might be part of the mechanism regulating the expression of MHC class II antigens on different cell types and therefore immunological responsiveness.

Our experiments demonstrate that regulation of MHC class II antigen expression on endothelial cells and on Langerhans cells of the skin is different, the former being abrogated by cyclosporin A, with the latter being unaffected. The consequence of this difference in the allograft situation will depend on the species. In the rat model it can be assumed that the donor passenger cells will be replaced by recipient cells. Because only

Table 1 Expression of MHC class II antigens in skin and kidney biopsies from dogs, before and during treatment with cyclosporin A

	Kidney		Skin	
	Glomerular endothelium	Peritubular endothelium	Capillary endothelium	Langerhans cells
Before treatment	+	+	+	+
During treatment	-	-	-	+

MHC class II antigen expression was studied with monoclonal antibodies using an indirect immunofluorescence technique. The presence or absence of antigens is indicated, respectively, by + and -.

passenger cells express MHC class II antigens in the rat kidney, the disappearance of these cells results in complete loss of donor-type MHC class II antigens, leading to long graft survival²². In man and dog, however, the expression of MHC class II antigens by endothelial cells prevents the disappearance of donor-type MHC class II antigens from an allograft. The present data show that removal can be achieved by cyclosporin A, thus suggesting an important new mode of action for this reagent in prolonging allograft survival.

We believe this to be the first demonstration that the expression of MHC class II antigens *in vivo* on endothelial cells is not a constitutive property of these cells. The expression *in vivo* on capillary endothelium in the canine kidney and skin is sustained by the immune system via lymphokines. The presence of MHC class II antigens on the capillary endothelial cells may be a consequence of the rheological characteristics of the vessels lined by these cells. The conditions for interaction between lymphocytes and endothelial cells, which are known to function as antigen-presenting cells^{23,24}, are most favourable on these MHC class II-positive capillary endothelial cells.

The variations in the expression of MHC class II antigens by endothelial cells as a result of products of the immune system, suggest new insights into the control of immune responses, and offer interesting opportunities for evaluating immune status in patients. Moreover, the abrogation of endothelial MHC class II antigen expression caused by cyclosporin A offers a new explanation for its immunosuppressive effect.

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Subtle structural alterations in the chains of type I procollagen produce osteogenesis imperfecta type II

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Although the perinatal lethal form¹ of osteogenesis imperfecta (OI type II) occasionally results from large rearrangements within the genes encoding type I collagen²⁻⁵, most mutations are far more subtle. The complexity of the human collagen genes⁶ precludes cloning and sequencing each gene from every patient, and we have therefore developed an approach to localizing mutations at the protein level. We report here that cells cultured from 15 infants with OI type II synthesized both normal type I procollagen and a form that was unstable, poorly secreted and excessively modified. Abnormal procollagen from different strains was overmodified to different extents. The patterns of overmodification we observed are best explained by mutations that disrupt the Gly-X-Y sequence of pro α chains, and thus alter the rate of propagation of triple helix from COOH-terminus to NH₂-terminus⁷. As a consequence, a given mutation allows overmodification of all three chains in a molecule NH₂-terminal to its position in the triple helix.

After pulse labelling with ³H-proline for 16 h, each of the 15 OI cell strains secreted pro α 1(I) and pro α 2(I) collagen chains with normal mobility and others with delayed mobility (Fig. 1a). The OI cells retained more pro α (I) and pro α 2(I) chains than control cells, and most of the intracellular procollagen consisted of chains with delayed mobility.

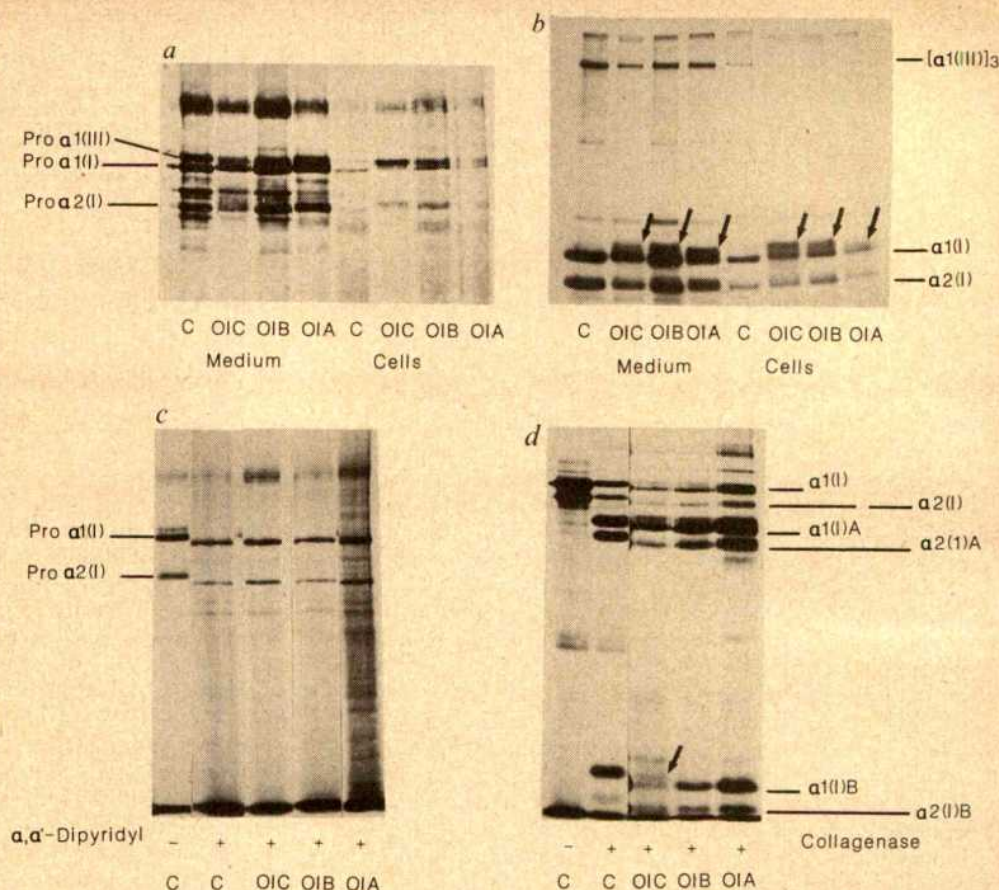
After treatment with pepsin, which removes the NH₂- and COOH-terminal propeptide extensions from type I procollagen molecules in triple helical conformation⁸, the OI α 1(I) and α 2(I) chains migrated as doublets (Fig. 1b). One chain from each doublet co-migrated with α 1(I) and α 2(I) chains from control cells and the other chain migrated more slowly than normal.

In the presence of α , α' -dipyridyl, which inhibits most of the intracellular modification of type I procollagen⁹, the OI cells synthesized only pro α 1(I) and pro α 2(I) chains, which co-migrated with control chains (Fig. 1c). Thus, the slow mobility of the abnormal pro α (I) and pro α 2(I) chains was apparently due to excessive modification, and the mutation in each OI cell strain described here was not a large insertion (or deletion^{10-14,29}) but was either a single amino-acid substitution or an insertion or deletion that we could not detect by SDS-polyacrylamide vertical slab gel electrophoresis (PAGE)¹⁵.

To determine the distribution of overmodification along the triple helix, we cleaved collagen-sized molecules asymmetrically with fibroblast collagenase (Fig. 1d). For some cell strains (for convenience these strains are designated group C), the abnormal α 1(I) and α 2(I) chains yielded a COOH-terminal B fragment and NH₂-terminal A fragment¹⁶, which were both overmodified. For other cell strains, only the NH₂-terminal A fragment was overmodified (groups B and A).

We further defined the extent of increased modification by the relative migration of each of four detectable CNBr peptides from abnormal α 1(I) chains (Fig. 1). For group C cell strains the entire triple helix was overmodified, for group B cell strains overmodification began near the fibroblast collagenase cleavage site, and for group A cell strains overmodification began

Fig. 1 a, Electrophoretic separation of pro α chains synthesized by OI and control cells. Three patient groups were identified based on the distribution of excessive post-translational modification within the triple helical domain (see Figs 2, 4). These groups designate biochemically distinguishable cell strains and do not correspond to the recently proposed clinical sub-classification of OI type II²⁵. Indeed, all patients described here fit the OI type IIA classification. There were seven cell strains in group A, three in group B and five in group C. The data from one representative of each group are shown. Two points emerge from these data. First, the electrophoretic mobilities of the pro α 1(I) and pro α 2(I) chains of some molecules are not normal. Second, there is considerable intracellular retention of abnormal pro α 1(I) and pro α 2(I) chains. The identity of bands with normal and delayed mobility was determined by CNBr peptide mapping (data not shown). **b**, Electrophoretic separation of α chains. The pro α chains synthesized by the OI type II cells were converted into two forms of α 1(I) and α 2(I), with one form of each chain migrating more slowly than normal and the other form co-migrating with the control (arrows indicate medium and cell-layer α 1(I) chains with delayed mobility). The delayed mobility of the abnormal α 1(I) and α 2(I) chains differed among the three groups; the delay was greatest for the abnormal α chains synthesized by cells from group C, intermediate for α chains from group B and least for α chains from group A. Resistance to peptic attack indicated that the abnormal pro α chains were incorporated into molecules that were triple helical in the conditions of digestion.



c, Electrophoretic separation of unmodified pro α chains. Only pro α 1(I) and pro α 2(I) chains that co-migrated with control chains were synthesized by the OI cell strains. The extent of hydroxylation of prolyl and lysyl residues and glycosylation of hydroxylysyl residues of type I procollagen synthesized by OI type II cells in culture has been measured directly by Steinmann *et al.*¹⁸ and by Bateman *et al.*²¹. Both studies found that prolyl hydroxylation was slightly increased over control levels and that lysyl hydroxylation and hydroxylysyl glycosylation were markedly increased, the overmodified pro α chains migrated more slowly on SDS-PAGE than their normal counterparts, and the difference in mobilities was abolished if the OI cells were pulse-labelled in the presence of α, α' -dipyridyl. **d**, Electrophoretic separation of α -chain fragments after cleavage with fibroblast collagenase. Type I collagen molecules synthesized by cell strains from group C yield A- and B-chain fragments that migrate as doublets (arrow indicates overmodified α 2(I)^B fragment). By contrast, only the A fragments from collagen synthesized by cell strains from groups B and A migrate as doublets while the B fragments co-migrate with the control.

Methods. **a**, Dermal fibroblast cell strains were established from explants of skin as described previously^{10,15}. Cells were plated at a density of 2.5×10^5 in 35-mm culture dishes and allowed to attach and spread overnight. The cells were preincubated for 2–4 h in 0.7 ml of serum-free Dulbecco's modified Eagle's medium (DMEM) containing $50 \mu\text{g ml}^{-1}$ of ascorbic acid. The medium with ascorbic acid was replaced with fresh medium and ascorbate and $[2,3,4,5\text{-}^3\text{H}]$ proline (101 Ci mmol^{-1} ; Amersham) was added for 17 h. The medium and cell layer were then collected separately into protease inhibitors¹⁵, dialysed into 1 mM ammonium bicarbonate containing the same inhibitors, and lyophilized. The dried samples were dissolved in electrophoresis sample buffer, and the proteins were denatured by boiling for 5 min. The proteins were separated in reducing conditions by SDS-PAGE with 2 M urea added to the buffers to enhance separation of pro α chains²⁶. Gels were processed for fluorography with ENHANCE (NEN). **b**, Medium and cell-layer proteins were collected as described above, dialysed into 0.5 M acetic acid, pH 2.4, and digested with pepsin ($50 \mu\text{g ml}^{-1}$; Worthington) for 12–16 h at 4°C. The reaction was stopped with pepstatin ($0.5 \mu\text{g ml}^{-1}$; Sigma). The samples were lyophilized and prepared for electrophoresis in denaturing but non-reducing conditions. **c**, Cells in culture were plated as before and then preincubated for 2 h in 0.7 ml of serum-free DMEM and 0.5 mM α, α' -dipyridyl (Sigma). The cells were then pulse-labelled with ^3H -proline in fresh medium plus α, α' -dipyridyl for 4 h. The cell-layer proteins were collected as described above and the constituent proteins were separated by SDS-PAGE in reducing conditions. Pro α chains from the medium of control cells labelled for 4 h in the absence of α, α' -dipyridyl have been separated in an adjoining lane for comparison. **d**, Collagen-sized molecules from the medium (after pepsin digestion) were dialysed into 50 mM Tris-HCl buffer pH 7.5, containing 10 mM CaCl_2 , then digested for 24 h at room temperature with fibroblast collagenase (final concentration $\sim 1.0 \mu\text{g}$ collagenase per $10 \mu\text{g}$ collagen). Differences in the relative migration of A- and B-chain fragments produced by the control and OI cell strains result from slight differences in the composition of SDS-PAGE.

NH_2 -terminal to this cleavage site within the domain of the triple helix defined by the α 1(I)CB7 peptide.

The increase in post-translational modification was accompanied by a decrease in thermal stability of the abnormal collagen molecules (Fig. 3). Molecules secreted by cells from group C had melting temperatures in the range 36–38°C, those secreted by cells from group B melted in the 38–39°C range, and those secreted by cells from group A melted in the 39–41°C range. The melting temperature of normal collagens secreted by OI cell strains and collagens secreted by control cell strains was 41.5–42.5°C. Thus, the abnormal OI molecules with the lowest melting temperatures were overmodified to the greatest extent.

These OI type II cell strains in culture synthesize unstable procollagen molecules that are excessively modified and poorly secreted. Because only some of the type I procollagen molecules from each strain are affected and because overmodification is

often asymmetrically distributed within the triple helical domain, we consider that the mutations harboured by these strains are in the genes encoding the pro α chains themselves. Their principal effect is to destabilize the triple helix of molecules incorporating a mutant chain.

Formation of a collagen triple helix requires a glycine in every third position¹⁷, and the structure is stabilized by hydrogen bonds that depend on hydroxyprolyl residues in the Y-position of repeating Gly-X-Y triplets. Hydroxylation of prolyl and lysyl residues begins on nascent chains and ceases when a stable triple helical conformation is achieved. Mutations which result in substitution for a glycine or in small deletions or insertions that disrupt the Gly-X-Y sequence could each produce an unstable triple helical domain and, as a consequence, cause the intracellular retention and excessive modification of affected molecules. As overmodification is often asymmetrically dis-

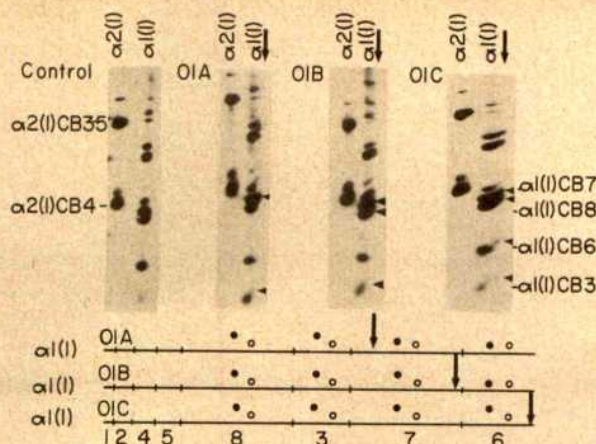


Fig. 2 CNBr peptides of $\alpha 1(I)$ and $\alpha 2(I)$ chains. We describe the migration of CNBr peptides derived only from $\alpha 1(I)$ chains because this chain yields four peptides from the region of the type I procollagen molecule that we are studying, whereas $\alpha 2(I)$ chains yield only two large peptides. The overmodified $\alpha 1(I)$ chain from group C OI cell strains yielded an array of CNBr peptides similar to the control, except that each peptide was delayed in mobility. The overmodified $\alpha 1(I)$ chain from group B cell strains yielded $\alpha 1(I)CB7$, $\alpha 1(I)CB8$ and $\alpha 1(I)CB3$ peptides with delayed mobility and an $\alpha 1(I)CB6$ peptide which co-migrated with $\alpha 1(I)CB6$ from the normal $\alpha 1(I)$ chain synthesized by the OI cells and from the control $\alpha 1(I)$ chain. The overmodified $\alpha 1(I)$ chain from group A cell strains yielded a single peptide in the region of $\alpha 1(I)CB7$ and $\alpha 1(I)CB8$ that migrated slightly more slowly than the normal $\alpha 1(I)CB7$ peptide, a peptide which migrated more slowly than $\alpha 1(I)CB3$, and a peptide that co-migrated with $\alpha 1(I)CB6$. We have shown by CNBr peptide mapping of these α chains after cleavage with fibroblast collagenase (see also ref. 15) that the large peptide spot migrating slightly more slowly than the normal $\alpha 1(I)CB7$ peptide consisted of overmodified $\alpha 1(I)CB7$ and $\alpha 1(I)CB8$ peptides (data not shown). Taken together with the results of fibroblast collagenase cleavage, we concluded that the abnormal type I procollagen molecules synthesized by the various OI cell strains were not overmodified to the same extent; for group C the entire triple helix was overmodified, for group B overmodification began near the fibroblast collagenase cleavage site, and for group A overmodification began NH_2 -terminal to this site within the domain of the triple helix defined by the $\alpha 1(I)CB7$ peptide. The line diagrams of the $\alpha 1(I)$ chains indicate the COOH-terminal end of the domain of increased post-translational modification (arrows). The vertical relationship between closed (OI) and open (normal) circles indicates the relative migration of CNBr peptides during electrophoresis.

Methods. Proteins were separated in the first dimension on 5% SDS-PAGE in denaturing but nonreducing conditions. The lanes were cut out and the proteins were digested in the gel with CNBr (100 mg ml⁻¹ in 70% formic acid; Sigma) for 2 h at room temperature^{10,15,27}. Each gel strip was washed with water for three 20-min periods, equilibrated for 20 min with sample buffer lacking SDS and urea, then placed horizontally over a 12.5% gel (second dimension) that contained no urea. The strips were overlaid with sample buffer containing dithiothreitol and 5% SDS, and the CNBr peptides were separated by electrophoresis.

tributed, we think that triple helix formation, which normally begins at the COOH-terminus of the helix and propagates toward the NH_2 -terminus⁷, proceeds at an appropriate rate until the region of the mutation is reached. Thereafter, the rate of formation of a stable triple helix is delayed and (because both pro $\alpha 1(I)$ and pro $\alpha 2(I)$ chains are excessively modified) all chains in abnormal molecules are available to the post-translational modifying enzymes for longer than normal^{15,18-21}. Assuming heterozygosity^{15,18} and the random assortment of nascent pro α chains in the rough endoplasmic reticulum as pro $\alpha 1(I)$ and pro $\alpha 2(I)$ chains associated to form trimers, only a portion of the procollagen molecules synthesized would be expected to contain a mutant chain.

These concepts are supported by studies which showed that a cysteine substitution within the triple helix of $\alpha 1(I)$ chains resulted in the overmodification, intracellular retention and instability of all molecules that contained the mutant chain¹⁸. Sequence analysis (D. Cohn, B. Steinmann, R. E. Gelinas and P. H. Byers, manuscript in preparation) has identified a single base substitution in a glycine codon that results in a Gly $\rightarrow$ Cys substitution 27 amino acids from the COOH-terminus of $\alpha 1(I)$ chains encoded by this allele. As the entire length of the triple helix of abnormal molecules was shown to be overmodified, the

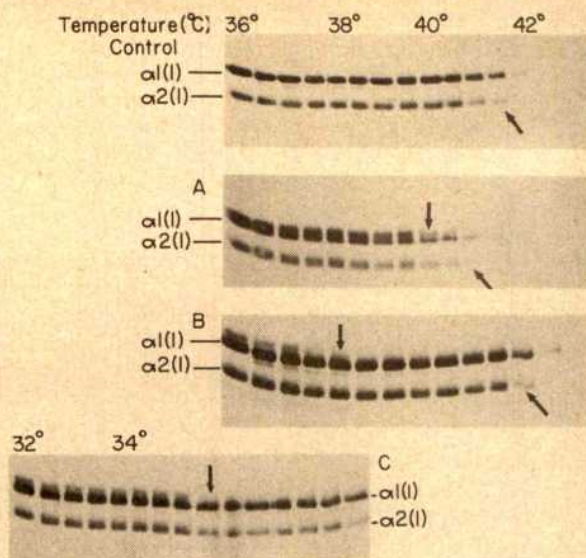


Fig. 3 The thermal denaturation temperature (T_m) of collagens secreted by representatives of OI type II cells in groups A, B and C.

Methods. Pepsin-digested procollagens from the medium were dissolved in 700 μ l of 0.4 M NaCl, 0.01 M Tris-HCl, pH 7.4. The samples were placed in a water bath at 30 °C and then warmed to 43 °C at a rate of 5 °C h⁻¹. Aliquots (36 μ l) were removed at 0.5 °C increments, rapidly cooled to 20 °C and then digested with trypsin (100 μ g ml⁻¹ final concentration; Worthington) for 2 min at 22 °C²⁸. The reaction was stopped by adding phenylmethanesulphonyl fluoride and SDS to achieve a final concentration of 2 mM and 2%, respectively. The whole mixture was placed in boiling water for 3 min. The proteins were separated by electrophoresis on 5% SDS-polyacrylamide gels containing 0.5 M urea. Fluorograms were quantitated by scanning gel densitometry. The slanted arrows below the top three fluorograms indicate the T_m of normal collagen molecules; the vertical arrows indicate the T_m of each abnormal molecule. In a separate experiment (data not shown) we have shown that the T_m of normal collagen secreted by the representative cell strain from group C was 41.5 °C.

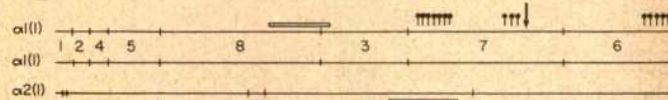


Fig. 4 Nature and location of mutations in the α chains of type I collagen synthesized by the cells from 17 patients with type II osteogenesis imperfecta. Circles indicate the sites at which increased post-translational modification begins in type I collagen molecules from 15 OI type II cell strains for which the mutation does not change the apparent length of pro α chains. Although arrayed along the $\alpha 1(I)$ chain, our data do not distinguish between mutations in $\alpha 1(I)$ or $\alpha 2(I)$ chains. The arrow indicates the fibroblast collagenase cleavage site. Bars indicate the approximate size and location of the deletion from $\alpha 1(I)$ and $\alpha 2(I)$, respectively, in two OI type II cell strains discussed previously^{10,11,22}.

position of the mutation in this cell strain is correlated precisely with the extent of overmodification. It is also notable that the abnormal type I procollagen molecules synthesized by OI cell strains harbouring large deletions are also overmodified, and the extent of overmodification can be correlated with the position of the mutation in the molecule.

Mutations that do not change the apparent length of the pro α chains of type I procollagen account for most of the mutations which give rise to the OI type II phenotype (Fig. 4). To date, cell strains from only three infants with OI type II have been shown to synthesize procollagen molecules that harbour deletions from either pro $\alpha 1(I)$ or pro $\alpha 2(I)$ ^{10-14,22,29}. In contrast, the 15 cell strains described here, 8 others described in detail elsewhere^{15,18,21} and 43 others that we have partially characterized (unpublished) synthesized molecules in which the mutation does

not result in a detectable change in pro α chain length. While the nature and location of these mutations will have to be determined by gene or messenger RNA sequence studies, mapping the sites of structural alterations in mutant proteins augments S₁ nuclease mapping strategies^{23,24} and identifies a small region within a large collagen gene for detailed study.

We thank the many physicians and families who provided us with skin biopsies from infants with OI type II, Dr Eugene Bauer for providing the fibroblast collagenase, and Nancy Higgins and William Dickie for preparing the manuscript. This work was supported in part by grants from the NIH (AM-21557, AM-07171, and GM-15253) and by a Clinical Research Grant (6-298) from the March of Dimes Birth Defects Foundation. J.B. is the recipient of an NIH individual fellowship award (AM-07171); P.H.B. was an Established Investigator of the American Heart Association during the tenure of this study.

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Sliding distance of actin filament induced by a myosin crossbridge during one ATP hydrolysis cycle

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Muscle contraction results from a sliding movement of actin filaments induced by myosin crossbridges on hydrolysis of ATP^{1,2}, and many non-muscle cells are thought to move using a similar mechanism³⁻⁵. The molecular mechanism of muscle contraction, however, is not completely understood^{6,7}. One of the major problems is the mechanochemical coupling at high velocity under near-zero load⁸⁻¹³. Here, we report measurements of the sliding distance of an actin filament induced by a myosin crossbridge during one ATP hydrolysis cycle in an unloaded condition. We used single sarcomeres from which the Z-lines, structures which anchor the thin filaments in the sarcomere, had been completely removed by calcium-activated neutral protease (CANP)¹⁴ and trypsin, and measured both the sliding velocity of single actin filaments along myosin filaments and the ATPase activity during sliding. Our results show that the average sliding distance of the actin filament is ≥ 600 Å during one ATP cycle, much longer than the length of power stroke of myosin crossbridges deduced from mechanical studies of muscle, which is of the order of 80 Å (for example, ref. 15).

Myofibrils were prepared from glycerinated crab (*Portunus trituberculatus*) leg muscle. Because the crab muscle has a long sarcomere (~ 7 μ m; thin and thick filaments are 2.7 and 4.8 μ m long, respectively), the sarcomere shortening takes a long time and the shortening velocity can be measured using an optical microscope. Figure 1a, b shows phase contrast and fluorescence images, respectively, of a myofibril labelled with phalloidin tetramethylrhodamine (PDTMR). In Fig. 1b, thin filaments only are bright because PDTMR specifically binds F-actin¹⁶. Figure 1c is a fluorescence micrograph of single sarcomeres in myofibrils from which the Z-lines have been removed. When 5 mM ATP was added to the single sarcomeres in the absence of Ca²⁺ at 0°C, thin filaments were dissociated from the thick filaments and dispersed into the solution. Single F-actin filaments labelled with PDTMR can be clearly observed in solution using the fluorescence microscope¹⁷. We confirmed that the thin filaments were completely dissociated from the A-band and the dispersed filaments single, not bundled (Fig. 1d). This point was also confirmed by electron microscopy. The treatment with CANP

and trypsin thus completely digested the Z-lines, leaving no structures to interconnect the thin filaments.

Using single sarcomeres without Z-lines, we next measured the sliding velocity of single thin filaments along thick filaments and the ATPase activity during sliding. Figure 2 shows sequential fluorescence micrographs of a single sarcomere without Z-lines during shortening at 5°C. The shortening velocity of PDTMR-labelled single sarcomeres was measured directly. Shortening was initiated by rapidly adding a small drop of ATP solution (final concentration 5 mM) to single sarcomeres which had sedimented and weakly adhered to the surface of a glass cell in rigor solution containing an ATP regenerating system and 0.2 mM CaCl₂ (Fig. 2). Thin filaments immediately started to slide into the H-band and met those from the opposite side at the centre of the H-band (M-line) 0.20-0.25 s after ATP addition. The velocity decreased but the shortening continued until the filaments had overlapped. The sliding velocity (V_s) of thin filaments along thick filaments in the H-band was 4.7 ± 0.5 μ m s⁻¹ at 5°C. The shortening velocity of single sarcomeres was also measured by monitoring the increase in light-scattering intensity of the sarcomere suspension after rapidly mixing it with ATP using a stopped-flow apparatus (Fig. 3). The shortening velocity obtained from the initial rise in the light-scattering intensity (5.3 ± 0.5 μ m per half-sarcomere per s at 5°C) agreed well with that measured directly under a fluorescence microscope (Table 1). The shortening velocity of the sarcomeres was about 1.7 times higher than the maximum shortening velocity of a muscle fibre under the same conditions (3.0 ± 0.2 μ m per half-sarcomere per s at 5°C). This does not result from the removal of the Z-lines because the shortening velocity of myofibrils before the removal was 1.5-2 times faster than this. Arata *et al.* have reported that the shortening velocity of rabbit psoas myofibrils (>10 μ m per half-sarcomere per s at 0°C) is also much higher than the maximum velocity of muscle fibres (<2 μ m per half-sarcomere per s)¹⁸. These high values do not result from the fact that shortening was initiated by adding ATP to actomyosin rigor complexes in myofibrils, because the shortening velocity was the same when initiated by adding Ca²⁺ to relaxed myofibrils. Therefore, these high velocities probably result from the removal of some connective materials^{19,20} during preparation of the myofibrils. Such a high velocity is also seen in proteoplasmic streaming in *Nitella* cells³ and movement of small organelles along actin cables in *Chara* cells²¹ (60-80 μ m s⁻¹ at room temperature) which are believed to be driven by the actin-myosin sliding movement.

Figure 3 shows the time course of ADP liberation from single sarcomeres without Z-lines after they had been rapidly mixed with ATP at 5°C under the same conditions as those used for

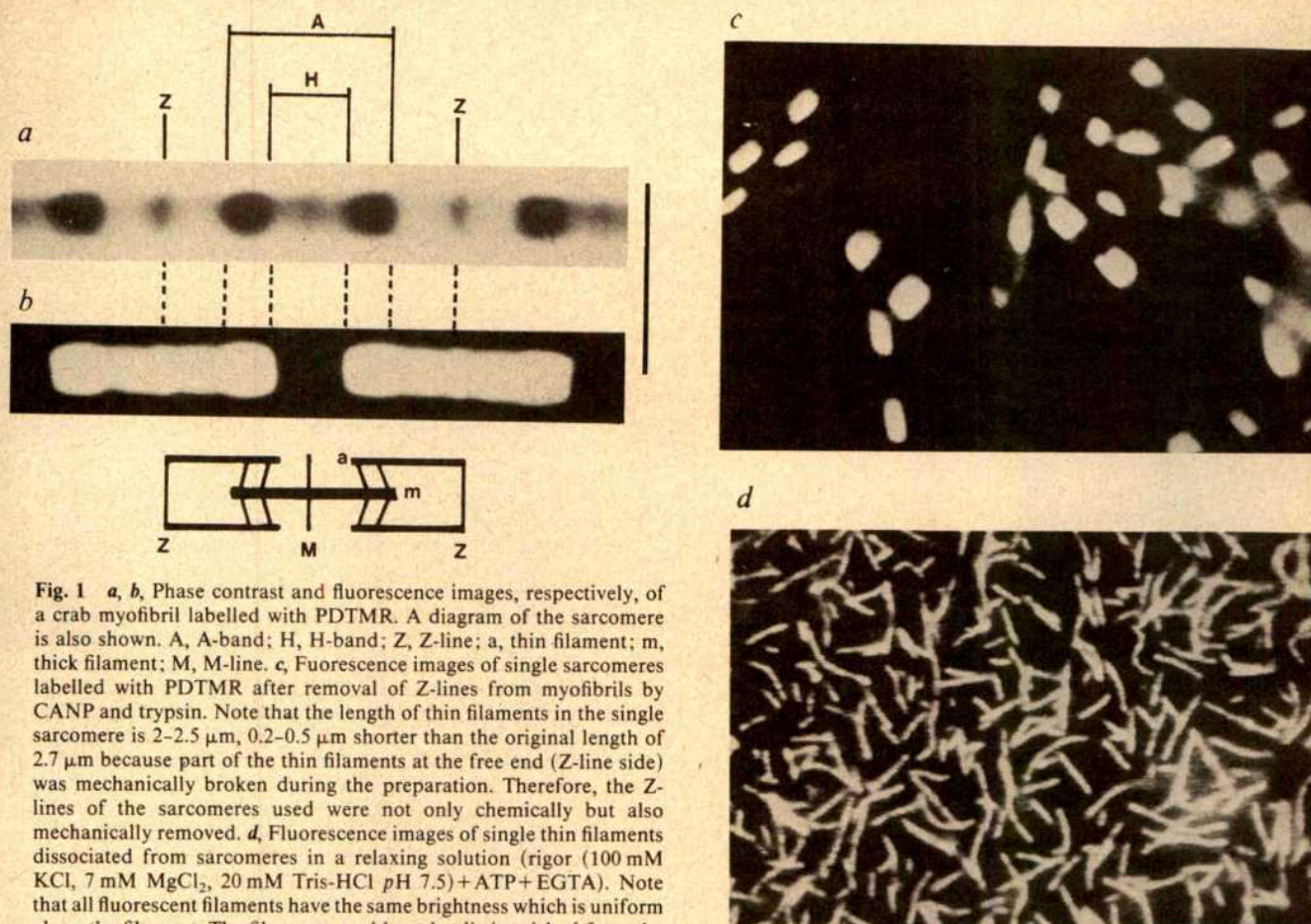


Fig. 1 *a, b*, Phase contrast and fluorescence images, respectively, of a crab myofibril labelled with PDTMR. A diagram of the sarcomere is also shown. A, A-band; H, H-band; Z, Z-line; a, thin filament; m, thick filament; M, M-line. *c*, Fluorescence images of single sarcomeres labelled with PDTMR after removal of Z-lines from myofibrils by CANP and trypsin. Note that the length of thin filaments in the single sarcomere is 2–2.5 μm , 0.2–0.5 μm shorter than the original length of 2.7 μm because part of the thin filaments at the free end (Z-line side) was mechanically broken during the preparation. Therefore, the Z-lines of the sarcomeres used were not only chemically but also mechanically removed. *d*, Fluorescence images of single thin filaments dissociated from sarcomeres in a relaxing solution (rigor (100 mM KCl, 7 mM MgCl_2 , 20 mM Tris-HCl pH 7.5) + ATP + EGTA). Note that all fluorescent filaments have the same brightness which is uniform along the filament. The filaments could not be distinguished from the fluorescence images of single thin filaments purified from rabbit skeletal muscle. Scale bars, 5 μm .

Methods. Myofibrils were prepared from glycerinated crab muscle and homogenized for 5 min in rigor solution with a Waring blender. CANP was obtained from the crab muscle and purified by the method of Ishiura *et al.*³². CANP specifically digests the Z-lines and hardly affects other protein^{14,33}. Thin filaments (F-actin) in myofibrils or sarcomeres were specifically labelled with PDTMR by immersing them in rigor solution containing $\sim 1 \mu\text{M}$ PDTMR for a few minutes at 0 °C. Treatment with PDTMR do not affect ATPase activity in the presence of Ca^{2+} (ref. 34). Fluorescent myofibrils and sarcomeres were observed with an inverted microscope (Zeiss IM 35) equipped with epifluorescence optics, a Zeiss Neofluor $\times 100$ objective (oil immersion, NA, 1.3), a 100 W mercury arc lamp and a Zeiss rhodamine filter ($\lambda_{\text{excitation}} = 540 \text{ nm}$; $\lambda_{\text{emission}} = 580 \text{ nm}$). Fluorescent single thin filaments were observed with a laser fluorescence microscope system described elsewhere³⁵. The fluorescent images were recorded on videotape with a high-sensitivity television camera (Ikegami SIT camera CTC-900; time resolution 17 ms) and a video recorder (Victor CR 6650L).

Table 1 Sliding distance of thin filament induced by a myosin crossbridge during one ATP cycle

Temperature (°C)	Sliding velocity of thin filament, V_s ($\mu\text{m s}^{-1}$)		ATPase activity, V_{ATP} (ADP mol per myosin mol s^{-1})*	Sliding distance of thin filament per ATP cycle, M (Å)†	
	Light scattering	Fluorescence microscope		Light scattering	Fluorescence microscope
5	5.3 $\pm$ 0.7(5)	4.7 $\pm$ 0.8(20)	1.0 $\pm$ 0.3(4)	680 $\pm$ 240	600 $\pm$ 230
10	12 $\pm$ 1.5(5)	—	—	—	—
15	20 $\pm$ 2.0(5)	—	4.1 $\pm$ 0.5(3)	650 $\pm$ 170	—

All observed values (V_s and V_{ATP}) are shown as mean $\pm$ half-range. Numbers in parentheses indicate the number of measurements. Values calculated from our measurements (M) are shown as the mean $\pm$ maximum possible error ($\delta M = |(\partial M / \partial V_s) \delta V_s| + |(\partial M / \partial V_{\text{ATP}}) \delta V_{\text{ATP}}|$).

* ATPase activity of each myosin molecule during sarcomere shortening was obtained as $V_{\text{ATP}} = V_s \times (W/C) \times 2L_0 / (L_0 + L)$, where V_s is ATPase activity of sarcomere shown as mol ADP per g sarcomere per s; C , myosin content (by weight) of a sarcomere; W , relative molecular mass of myosin (4.8×10^5 , ref. 28); L , length of the overlap region between thin and thick (crossbridges) filaments per half-sarcomere before shortening; L_0 , the length at full overlap of thick and thin filaments. ATPase activity in the non-overlap region (in the relaxed state) was neglected because it was expected to be >20 times smaller than that in the overlap region during shortening. The average values of L_0 and L of sarcomeres used here were 2.3 and 1.1 μm , respectively. C was calculated to be 0.35 by comparing the area of the myosin heavy chain of the sarcomere with that of purified myosin, the weight of which was found according to Arata *et al.*²⁹, on SDS gels.

† The sliding distance of thin filaments during one ATP cycle (lower limit) was obtained according to the equation shown in the text from the sliding velocity (V_s) and the ATPase activity (V_{ATP}). The average number of myosin molecules in half a thick filament overlapping with thin filaments during sliding, N_m , was obtained as $N_m = N_m \times (L_0 + L) / 2L_0$ ($= 640 \times (2.3 + 1.1) / 4.6 = 470$). The number of myosin molecules in half a thick filament, N_m , was obtained as $N_m = (L_0 / P_m) \times n$ where P_m is the myosin subunit repeat (14.3 nm; Namba *et al.*²²); and n , number of myosin molecules per repeat ($= 4$; Wakabayashi^{30,33}). The value of n was also determined to be ~ 4 (3.8–4.3) by SDS polyacrylamide gel electrophoresis according to Tregar and Squire³¹.

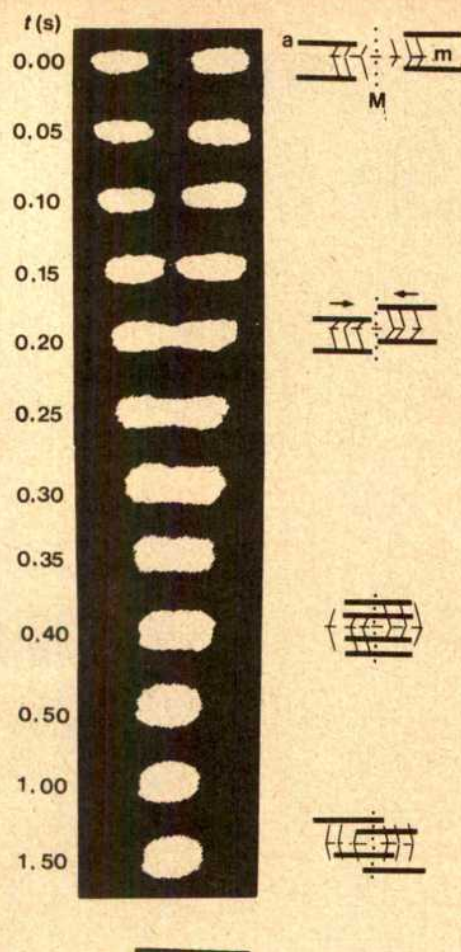


Fig. 2 Sequential fluorescence micrographs of a PDTRM-labelled sarcomere without Z-lines during shortening at 5°C. Times (t) are shown in s. Scale bar, 5 μ m. Shortening was initiated by rapidly adding ATP solution from a small pipette at $t = 0$ s to the suspension of sarcomeres on a glass slide enclosed with a bank of glue. The bathing solution (rigor + 0.2 mM CaCl_2) contained 0.2% β -mercaptoethanol to suppress photobleaching. At $t \geq 0.5$ s only a small spot, probably the overlap region, could be seen at the set level of brightness; the image of the ends of filaments became too vague to be visualized. Sample temperature was controlled by attaching a metal rod connected to a bath cooler to the stage of the inverted microscope.

the measurement of shortening velocity. The ATPase activity during shortening at 5°C was 1.0 ± 0.3 mol ADP per mol myosin s^{-1} at 2 mM ATP and decreased to 0.13 ± 0.02 mol ADP per mol myosin s^{-1} after completion of the shortening.

The average sliding distance of a thin filament induced by hydrolysis of 1 mol ATP was calculated from the sliding velocity of the thin filament and the ATPase activity during sliding. The average number of myosin molecules in half a thick filament overlapped with the thin filaments during the shortening, $\bar{N}_m$, is 470 (see Table 1). The ATPase activity during the sliding, V_{ATP} , is 1 s^{-1} at 5°C (Table 1) and the ratio of thin to thick filaments, r , is 6 (ref. 22). Therefore, the number of ATP cycles on one thin filament per s, N_{ATP} , was calculated as $N_{\text{ATP}} = (\bar{N}_m \times V_{\text{ATP}})/r = (470 \times 1 \text{ s}^{-1})/6 = 78 \text{ s}^{-1}$. The sliding distance of the thin filament during one ATP cycle, M , is calculated as $M = V_s/N_{\text{ATP}} = 4.7$ (or 5.3) $\mu\text{m s}^{-1}/78 \text{ s}^{-1} = 600$ (or 680) Å assuming that the ATP cycles occur in turn without overlapping. This value should be the lower limit because the ATP cycles sometimes occur simultaneously, in which case M would become larger. When the temperature was increased to 15°C, a similar high value of M was obtained (Table 1). Thus, the sliding distance of the thin filament induced by the myosin crossbridge during one ATP hydrolysis cycle in the unloaded

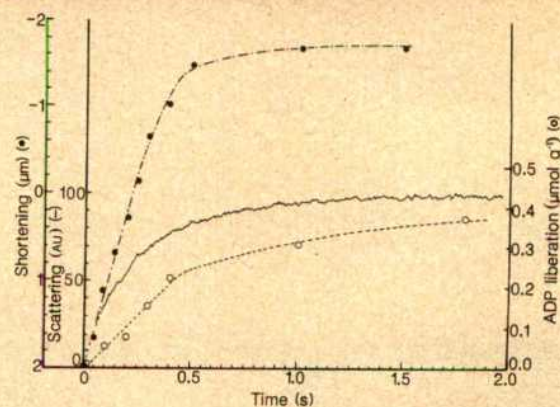


Fig. 3 Time course of shortening, light-scattering intensity and ADP liberation during contraction of single sarcomeres without Z-lines.

Methods. Closed circles, extent of shortening observed directly by an inverted fluorescence microscope (see Fig. 2). Ordinate, width between the tops of thin filaments on both sides of the sarcomere (H-band). After these filaments had overlapped, their width was measured between their tail ends and shown as negative values. Solid line, intensity of light scattering from a suspension of single sarcomeres at 90° and $\lambda = 460 \text{ nm}$. The light-scattering intensity increased following an exponential equation consisting of two time constants and then decreased by >10 times slower than the fastest phase (not shown). Assuming that the fastest component of the increase in the light-scattering intensity arose from an increase in the overlap between thin and thick filaments at the A-band and the second from the overlap between the thin filaments from the two opposite sides, the sliding velocity of thin filaments was calculated as $V = (\frac{1}{2}l)/\tau_1 = (\frac{1}{2} \times 2.3 (\pm 0.2) \mu\text{m})/0.22 \text{ s} = 5.2 (\pm 0.5) \mu\text{m s}^{-1}$, where τ_1 is the fastest time constant and l the width of the H-band. l was measured with a fluorescence microscope after labelling with PDTRM. The suspension of sarcomeres was rapidly mixed with ATP and the time course of change in light intensity measured with a stopped-flow spectrophotometer (Union Giken RA-401). Open circles, ADP liberation, measured by the method of Arata *et al.*¹⁸. The ATPase reaction was initiated by rapidly adding a small amount of concentrated ATP solution to the 20 mg ml^{-1} suspension of sarcomeres in rigor solution containing 0.2 mM CaCl_2 , vigorously stirred and stopped by adding HCl (final concentration 0.3 M). ATP and HCl solutions were added using a small syringe connected to an electric solenoid. The ATPase reaction of the sarcomeres (width of H-band = $2.3 \pm 0.2 \mu\text{m}$) was coupled with 15 mg ml^{-1} pyruvate kinase and 2 mM phosphoenolpyruvate to regenerate ATP. The ATPase activity was determined from the rate of pyruvate liberation, measured by the method of Reynarn *et al.*³⁶. The maximum activity of pyruvate kinase (Sigma) was $50 \mu\text{mol mg}^{-1} \text{ min}^{-1}$ in rigor solution at 5°C. When the ATPase activity of sarcomeres of 20 mg ml^{-1} is $20 \mu\text{M s}^{-1}$ (1.4 per mol ADP per mol myosin per s, see Table 1), the lag between the ATP hydrolysis and ATP regeneration reactions in the presence of 15 mg pyruvate kinase per ml is estimated to be only 0.015 s, taking into account that the Michaelis constant of ADP for pyruvate kinase is 0.2 mM³⁶. The protein concentration of the sarcomere was measured by the biuret method and the absorbance at 280 nm was determined after treatment with 1 M NaOH for 24 h at 20°C (ref. 37). All experiments were performed at 5°C.

condition is much longer than the size of the power stroke of the crossbridge, 80 Å deduced from studies on isometric tension transients of muscle (ref. 15).

Because the Reynolds number (inertial force/viscous force) is extremely small for the movement of the thin filament ($\sim 10^{-5}$) or the relaxation time of the velocity of the thin filament after removal of the external force is $<1 \text{ ns}$ ($= m/\zeta = Dm/kT = (10^{-8} \text{ cm}^2 \text{ s}^{-1} \times 10^{-16} \text{ g})/(1.38 \times 10^{-23} \text{ J K}^{-1} \times 300 \text{ K}) = 10^{-10} \text{ s}$, where m is the mass of the thin filament; ζ , the friction constant; D , translational diffusion constant; k , Boltzmann constant; and T , temperature)²³, the movement of the thin filament induced by the myosin crossbridge decays immediately ($<1 \text{ ns}$) after its detachment and the thin filament cannot be moved by an inertial force. The possibility of the movement of several other filaments

hydrodynamically induced by the movement of a thin filament is expected to be very low for two reasons; first, the myosin heads and filament backbone that occupy most of the space in the sarcomere^{22,24} are expected to almost completely abolish the flow of the solution; second, even if the flow of solution is induced, it would almost be abolished because the directions of the flow from two sides of the sarcomere oppose each other. It is also unlikely that the high sliding velocity of the thin filaments in the sarcomere results from electrostatic interactions between them, as increasing the concentration of free Mg^{2+} from 1 to 7 mM did not increase the shortening velocity, although the attractive force among the thin filaments should be stronger at higher concentration of divalent cations²⁵. In fact the shortening velocity decreased by 10% at 100 mM KCl and 30% at 50 mM KCl. Furthermore, the fact that the elastic modulus of thin filaments obtained from analysis of their thermal bending motion in a myosin-free ghost fibre²⁶ is almost the same as that when they are independent in solution¹⁷ strongly suggests that the electrostatic interaction among thin filaments in the sarcomere is very weak.

How can such a long sliding distance of the thin filament per one ATP hydrolysis cycle be reconciled with other measurements? One possibility is that more crossbridge than ATP hydrolysis cycles occur during a transient phase after the start of unloaded shortening¹¹⁻¹³ or during steady state unloaded shortening^{8-10,27}. If there are six or more crossbridge cycles per ATP cycle, the sliding distance obtained here would be similar to that deduced from mechanical studies, 80 Å (ref. 15). The experimental data (Table 1) showed that the number of ATP hydrolysis cycles during the shortening period was about a quarter of the total number of myosin heads. Therefore, it is possible that only a fraction of crossbridges complete the ATP hydrolysis cycle during the shortening period itself. It is unlikely, however, that many of the remaining crossbridges could do work during shortening and hydrolyse ATP after shortening, because the ADP release after shortening is small (Fig. 3).

Another possibility is that when a crossbridge hydrolyses an ATP molecule and produces thin filament sliding, some free energy of hydrolysis is transferred passively to another crossbridge attached to the thin filament; that is, the first crossbridge does work on a second. Then, the second crossbridge produces sliding of the thin filament and again does work on a third. Such a cycle would occur repeatedly during one ATP hydrolysis cycle. This kind of mechanism, however, would require a very regular and directional order of crossbridge attachment-detachment cycles on the thin filament, so that the possibility may not be completely excluded but seems unlikely.

We believe a more straightforward interpretation of our results is that the range of movement over which myosin crossbridge can attach to the actin filament during one ATP hydrolysis cycle under an unloaded condition is at least 600 Å.

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Alterations of the *hprt* gene in human *in vivo*-derived 6-thioguanine-resistant T lymphocytes

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Investigations into the extent and significance of somatic gene mutations occurring *in vivo* in humans have been hampered by the lack of a means of unambiguously defining the mutational origin of *in vivo*-derived variant cells. Several years ago we proposed that 6-thioguanine-resistant T lymphocytes, present at low frequencies in human peripheral blood, might be useful markers of *in vivo* somatic mutation^{1,2}. We and others have since described methods for the isolation and study of these unusual cells³⁻⁵. The thioguanine-resistant T cells stably lack hypoxanthine-guanine phosphoribosyltransferase (HPRT) activity, suggesting that they are somatic equivalents in normal individuals to cells from individuals with the X-chromosomal *hprt* Lesch-Nyhan germinal mutation^{6,7}. We now report that *in vivo*-derived thioguanine-resistant T-cell colonies from a single normal individual show a variety of *hprt* structural alterations, as determined by Southern blot analysis. This finding demonstrates unequivocally that these cells are genetic mutants and validates their use for fundamental and applied mutational studies in humans.

Human T cells were obtained from a healthy male donor and colonies were isolated from a clonal assay, as described by Albertini *et al.*³. Briefly, lectin-activated lymphocytes, mixed with T-cell growth factor and feeder cells, were inoculated into the wells of microtitre plates in the absence or presence of 10^{-5} M 6-thioguanine (6-TG). An average of 2 or 10^5 cells per well was inoculated into non-selected wells or 6-TG-selected wells, respectively. After 16 days, 118 of 960 non-selected wells and 24 of 960 6-TG-containing wells showed growing colonies. Cloning efficiencies, calculated by the Poisson relationship $P_0 = e^{-x}$ (where P_0 is the frequency of negative wells and x the average number of cells per well), were 6.6% and 0.025×10^{-5} in the absence and presence of thioguanine, respectively, yielding a 6-TG-resistant mutant frequency of 3.8×10^{-6} .

Twelve unselected (wild type) and 24 thioguanine-selected (mutant) colonies were isolated and subcultured; 9 unselected and 15 thioguanine-selected colonies achieved total cell numbers of $50-100 \times 10^6$ cells. All cells were positive for the surface marker T11, confirming their T-cell identities. HPRT enzyme activity⁸ in the nine unselected colonies ranged from 30 to 50 pmol inosine monophosphate produced per min per extract of 10^6 cells; $<10\%$ HPRT activity was detected in the 15 6-TG-selected colonies.

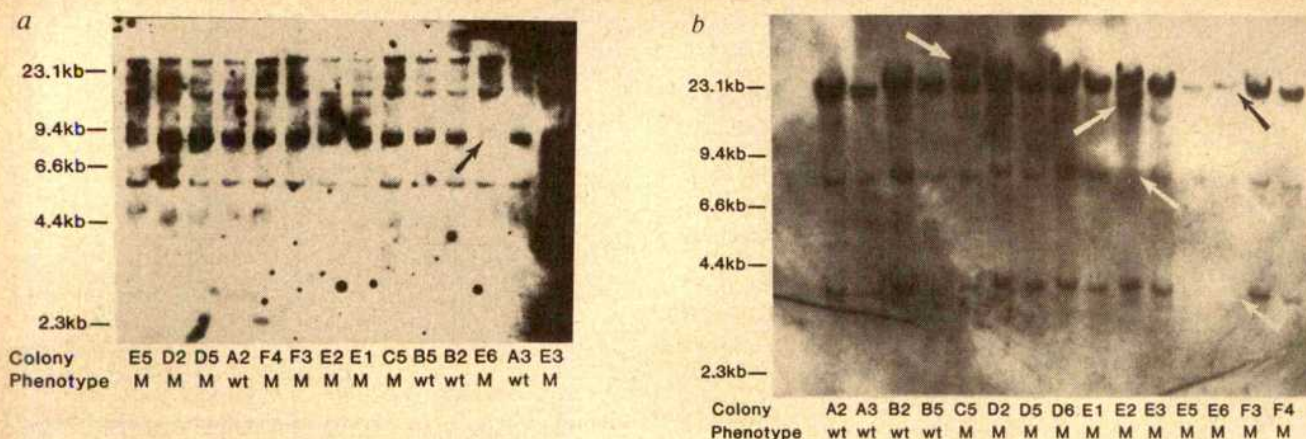


Fig. 1 Southern blots of DNA isolated from 4 (A2, A3, B2, B5) wild-type (wt) and 11 (C5, D2, D5, D6, E1, E2, E3, E5, E6, F3, F4) 6-TG-resistant mutant (M) T-cell colonies isolated from one individual and probed with a full-length *hprt* cDNA probe (pHPT31 insert⁹, given by Dr C. T. Caskey). The *hprt* insert of 947 bp contains at least 100 bp of sequence 5' to the initiation codon, the entire coding sequence and ~200 bp of the 3'-untranslated region. **a**, *EcoRI*-digested DNAs. Mutant E6 shows loss of hybridization to an 8.0- and an 8.3-kb fragment. **b**, *BamHI*-digested DNAs. Mutant C5 shows a new fragment at 35 kb, with a possible compensatory loss in the 22–25-kb region which is difficult to interpret because two *hprt* fragments and a cross-hybridizing autosomal fragment localize to this region. Mutant E2 shows a new fragment at 19 kb and loss of the 8-kb fragment. Mutant E5 shows loss of all fragments except the 25-kb autosomal fragment; however, this lane was underloaded. Mutant E6 has definitely lost hybridization to all four *hprt* fragments, although the 25-kb autosomal fragment remains. (Ethidium bromide staining and use of another, non-*hprt*-related probe demonstrated a full aliquot of DNA in all lanes except E5, clearly eliminating underloading as an explanation for fragment losses in E6).

Methods. To isolate the DNA, 15–20 × 10⁶ frozen cells for each colony were washed and resuspended in 1 ml T₁₀E₁ (10 mM Tris, 1 mM EDTA pH 8.0), then 4 ml TENS (25 mM Tris-HCl pH 8, 100 mM NaCl, 10 mM EDTA, 0.6% SDS) was added and the solution heated to 65 °C for 15 min. Proteinase K (0.5 mg) was added, and the mixture incubated at 37 °C overnight, then an additional 0.25 mg of proteinase K was added and digestion was continued for 3 h. The resulting solution was phenol-extracted twice, followed by chloroform/isoamyl alcohol (24:1) extraction, then ethanol precipitation and resuspension in T₁₀E₁. For the Southern blots, ~7.5 µg per lane of genomic DNA was digested with restriction enzymes, fractionated on a 0.7% agarose gel in TAE (40 mM Tris-acetate, 2 mM EDTA) buffer and transferred to nitrocellulose filters (Schleicher & Schuell). Prehybridization was for 5–16 h at 42 °C in Stark's solution¹², then 1–1.5 × 10⁶ c.p.m. ml⁻¹ of oligonucleotide-primed ³²P-labelled *hprt* insert was added and incubation continued at 42 °C for 16 h. Washing consisted of two 10-min washes in 2 × SSC, 0.1% SDS at room temperature and two 1-h washes in 0.1 × SSC, 0.1% SDS, the first at 50 °C and the second at 60 °C. Autoradiography was performed at –80 °C with Kodak XAR-5 film and Dupont lightning plus screens for 3 days.

Southern blot analysis was used to determine alterations of the DNA in the *hprt* genes of the 6-TG-resistant colonies. Genomic DNA of 4 wild type and 11 mutant colonies was isolated from frozen samples of 15–20 × 10⁶ cells. The DNA, cleaved with *EcoRI*, *HindIII*, *BamHI* or *MspI*, was probed with a 947-base pair (bp) full-length *hprt* complementary DNA clone⁹. This *hprt* cDNA hybridizes with four or five restriction fragments in genomic DNA, representing exons of the X-linked *hprt* gene, and also cross-hybridizes with three to four restriction fragments whose sequences have been localized on chromosomes 3, 5 and 11 (refs 10, 11).

On the *EcoRI* Southern blot (Fig. 1a), mutant E6 demonstrates a loss of probe hybridization to two fragments that migrate at 8.0 and 8.3 kilobases (kb) in the wild-type DNA. On the *BamHI* blot (Fig. 1b), mutants C5 and E2 show novel large fragments and E2 has also lost probe hybridization to a fragment at 8 kb. An *MspI* blot (Fig. 2) reveals a striking loss of fragment hybridization for mutant E6.

Using characterized subfragments of the full-length *hprt* cDNA clone, Caskey and co-workers mapped the 5'-untranslated, 5' coding, 3' coding and 3'-untranslated regions to specific restriction fragments generated by several endonucleases¹¹. Thus, it is possible to determine where alterations have occurred in the *hprt* DNA sequences of somatic 6-TG-resistant mutants by comparing the fragments absent in the mutants with the known restriction-fragment maps of the *hprt* gene^{10,11}. For the *BamHI*, *EcoRI* and *HindIII* blots, all of the missing or changed fragments reported here represent the X-linked *hprt* gene and not the autosomal cross-hybridizing sequences. However, as information on the chromosomal location and gene mapping of the *MspI* fragments has not yet been reported, definite statements on the gene alterations represented by changes in restriction fragment patterns with this endonuclease cannot be made.

Overall (see Table 1), and including data from other blots (not shown), four of the mutant colonies (D2, D6, E1 and E3) showed no restriction fragment alterations, compared with the four wild-type colonies (which, as expected, had identical patterns). Mutant E2 showed a reciprocal gain of hybridization to a novel fragment and a loss of hybridization to a wild-type *BamHI* fragment representing the 5' end of the gene. Mutant E6 demonstrated changes with all four of the enzymes tested, suggesting a deletion involving a considerable portion of the 3' end of the gene. Mutant C5 showed changes with two enzymes. *MspI* showed a gain of a large fragment, with corresponding loss of a small fragment; *BamHI* showed a gain of a 35-kb fragment with a possible loss of a 22–25-kb fragment (the migration of several fragments in the 22–25-kb region makes interpretation difficult). The blots for the remaining four mutants (D5, E5, F3 and F4) were difficult to score. Two mutants (D5 and F3) showed weak hybridization to a new 1.95-kb *MspI* fragment, perhaps as a consequence of incomplete digestion. For E5 and F4, there was insufficient DNA to test more than one or two enzymes.

The 11 6-TG-resistant mutant colonies described here were developed from progenitor mutant cells that arose *in vivo* in a single normal adult male. Thus, the different mutations do not represent a polymorphism among individuals but, rather, indicate that various molecular lesions can give rise to somatic mutations at this single locus within an individual.

Yang *et al.* demonstrates *hprt* DNA changes in 5 of 28 independent germinal Lesch-Nyhan mutations after digestion with four endonucleases¹⁰. By comparison, 3 of the 11 somatic mutants studied here (C5, E2 and E6) show obvious alterations in the *hprt* structural gene. Thus, at least four independent mutational events have occurred. However, this is certainly an underestimate for at least two reasons. First, two other colonies,

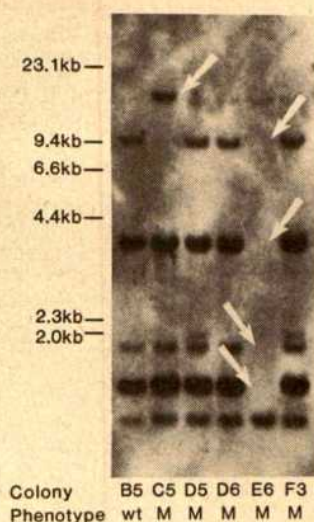


Fig. 2 Southern blot of DNA from one wild type (B5) and five thioguanine-resistant mutant (C5, D5, D6, E6, F3) colonies digested with *MspI* and probed with the *hprt* insert from pHPT31. Mutant C5 shows hybridization to a novel fragment at 16.5 kb and reciprocal loss of the 9.4-kb fragment. Mutant E6 shows loss of hybridization to the 9.4-, 3.8-, 1.8- and 1.35-kb fragments. The new faint fragments at 2.0 kb for D5 and F3 may be a result of partial digestion and are not considered to be new fragments. (Methods as for Fig. 1).

E5 and F4, show possible structural changes with one or more of the four endonucleases used. Analysis with additional endonucleases will probably define more colonies having detectable DNA alterations. Second, we do not know what proportion of the other mutants not showing gross rearrangements were a result of small deletions, duplications or point mutations. Further study is necessary to define the number of independent mutational events responsible for this collection of thioguanine-resistant mutants. However, the results presented here do constitute unequivocal evidence for the mutational origin of the thioguanine-resistant T cells that arise *in vivo* in the normal human.

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Table 1 Summary of restriction fragment changes in thioguanine-resistant mutant T-cell colonies

Mutant colonies	<i>Bam</i> HI (kb)	<i>Eco</i> RI (kb)	<i>Hind</i> III (kb)	<i>Msp</i> I (kb)
C5	+35 (-22 to 25)*			+16.5, -9.4
D2				ND
D5				
D6				
E1				ND
E2	+19, -8			ND
E3				ND
E5	(-22, -25, -8, -3.7)*	(-8.0, -8.3)*		ND
E6	-22, -25, -8, -3.7	-8.0, -8.3	-17	-1.35, -3.8 -1.8, -9.4
F3				
F4			(-40)*	

+ Indicates the presence of a novel restriction fragment; - indicates loss of hybridization to a wild-type restriction fragment. ND, not determined.

* Difficult to interpret.

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A hybrid recognition sequence in a recombinant restriction enzyme and the evolution of DNA sequence specificity

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Early attempts to generate new restriction specificities by recombination between allelic restriction-modification systems have been unsuccessful¹. Bullas *et al.*² succeeded in isolating a new specificity, SQ, in *Salmonella* that they interpreted as being the result of a recombination event between the parental strains, *Salmonella typhimurium* and *S. potsdam*, which encode the SB and SP restriction systems, respectively. This interpretation has recently been confirmed by DNA heteroduplex studies with the SB, SP and SQ structural genes³. We have determined the DNA sequences recognized by the SB and SP enzymes⁴ and found that, like all type I restriction sequences, they are split into two specific domains by a spacer of nonspecific sequence that, for both SB and SP, is 6 base pairs (bp) long. We have now determined the sequence recognized by the recombinant SQ enzyme and find that it is a hybrid between the SB and SP sequences, containing one specific domain from each parental strain. This result implies that each of the two specific domains is recognized by a physically distinct part of the enzyme.

The SQ enzyme was purified from the strain L4004 (ref. 2) by methods used previously for SB and SP⁴. Its recognition sequence was determined using a computer-aided strategy described in detail elsewhere⁴. The method depends on the fact that type I restriction enzymes are also modification methylases and thus can be used to introduce radioactive methyl groups into their recognition sites in sequenced DNA molecules. The radioactive label is then mapped to small regions of the DNA by restriction analysis and fluorography and computer analysis is used to find DNA sequences common to all labelled regions and absent from all others.

We screened various different sequenced DNA molecules for the presence of SQ sites by *in vitro* methylation with [³H-Me]-S-adenosyl methionine. DNA molecules that had no SQ sites were pBR322, pBR325, pSHI44, pFR100, ØX174, G4, M13 and simian virus 40. Phage λ DNA, which has been reported not to be restricted by the SQ system², was a substrate for the enzyme. In our hands SQ does restrict, with plating efficiencies ranging from 10⁻¹ to 10⁻² in different experiments. Mapping experiments showed that λ DNA contains a single site for the enzyme, which

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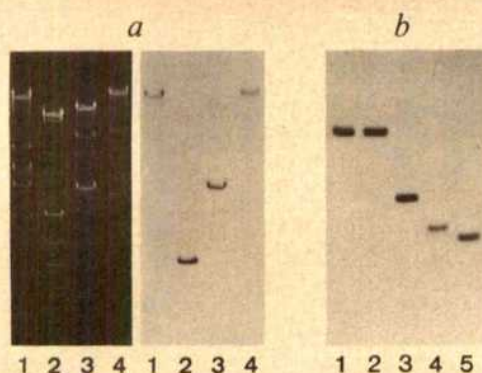


Fig. 1 Mapping of the SQ site in phage λ DNA. The DNA was methylated with purified SQ enzyme and [$^3\text{H-Me}$]-S-adenosyl methionine (Amersham, 62–74 Ci mmol $^{-1}$) as described earlier⁴. The labelled DNA was then cleaved with different type II restriction enzymes and the fragments were separated on either a 1% agarose gel (a) or an 8% polyacrylamide gel (b). The gels were photographed after staining with ethidium bromide and then fluorographed. a, Mapping of the SQ site with enzymes that cut the DNA infrequently. The left panel shows the stained gel, the right panel the fluorogram. The enzymes used and the extent of the labelled fragments are: lane 1, *EcoRI*, 1–21,226; lane 2, *AccI*, 1–2,190; lane 3, *AvaI*, 1–4,720; lane 4, *HindIII*, 1–23,120. b, Fine mapping of the site and confirmation of the sequence. Only the fluorogram is shown. Lane 1, *HaeIII*, 403–469; lane 2, *HaeIII* + *RsaI*, the same fragment is labelled as in lane 1, even though *RsaI* has a site at position 439; lane 3, *BstNI* + *HaeIII*, 425–469; lane 4, *BglII* + *TaqI*, 415–451; lane 5, *BstNI* + *FnuDII*, 425–459. We conclude that a SQ site is present between positions 425 and 451.

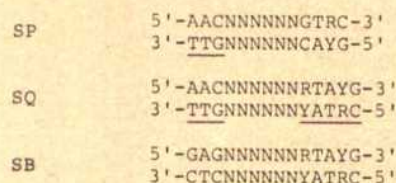


Fig. 2 The recognition sequences of SP, SB and SQ. Homologies between the sequences are indicated by bars.

we first mapped to the left 2,190 bp of the DNA molecule (Fig. 1a) and then, using combinations of fine-cutting enzymes, to a 26-bp stretch extending from positions 425 to 451 of the λ DNA sequence⁵ (Fig. 1b). Another SQ site was found and mapped to a small region of the yeast *PHO5* gene⁶ (not shown). The two regions that contained SQ sites, together with all the sequences that did not, were subjected to computer analysis as described above. Most sequences were taken from the DNA sequence databank of the European Molecular Biology Laboratory (EMBL). This search failed to find a non-degenerate sequence that could be a candidate for the SQ recognition site, but further searches found a single sequence with one degeneracy that fitted the data 5'-AACNNNNNNRTACG-3', where N is any nucleotide and R is either purine. The experiment shown in Fig. 1b, lanes 1 and 2, confirms this sequence. In phage λ , G is present in the position indicated as R and the resulting 5'-GTAC-3' is an *RsaI* recognition site. Figure 1 shows that *RsaI* does not cut this site in SQ-modified DNA, indicating that SQ has methylated the DNA within the site.

We then searched the EMBL DNA sequence databank for other DNA molecules containing the putative SQ site and variants of it and found that the plasmids p3201 (ref. 7) and *colE1* (ref. 8), phage T7 DNA⁹ and the yeast *CYTcI* (ref. 10) gene were suitable for further analysis. The SQ sites in all of these DNA molecules were labelled and mapped (not shown) and the results are summarized in Table 1. One further degeneracy in the sequence was found and the SQ recognition site is 5'-AACNNNNNNRTAYG-3', where Y can be either pyrimidine. Sequences that had pyrimidines in the position marked by R or purines in the position marked by Y were not

Table 1 Compilation of SQ recognition sequences

DNA	Position	Sequence
λ	430	GAAGTATTGAGTACGA
PHO5	1,612	TAACTGCCGAATACGT
CYT cI	1,401	AAACAATTGAGTATGC
ColE1	3,439	CAACAGGACTATATGC
T7	19,953	GAAGTGGACCATATGT
T7	31,459	CAACGTTGATGTACGC
p3201	1,142	TAACTGCCGTATATGT
p3201	2,495	GAAGTGGATGGTATGC
Consensus:		AACNNNNNNRTAYG

substrates for the enzyme. All four nucleotides can be found flanking the sequence and at all positions of the nonspecific spacer (Table 1).

Figure 2 compares the recognition sequence of SQ with those of its parents, SB and SP. SQ has the AAC domain of SP and the RTAYG pentanucleotide domain of SB. It is clear that the recombination event that generated SQ took place within the region encoding the part of the polypeptide that is involved in sequence recognition.

The allelic type I restriction enzymes found in *Escherichia coli* and *Salmonella* spp. are complex enzymes, and DNA recognition is a function of just one of their subunits, the product of the *hdsS* gene (reviewed in ref. 11). Several *hdsS* genes have been sequenced¹² and the sequences are surprisingly non-homologous, only two short regions, one in the middle of the gene and the other at the 3' end, being highly conserved. The recombination that yielded SQ took place in the central conserved region³. It is not known whether the parts of the polypeptide that bind the recognition sequence are encoded by this region or by the nonconserved sequences on either side.

Recombination between *hdsS* genes has the potential for generating any combination of specific sequence domains and thus of creating new sequence specificities. There is some indication that this may have happened naturally in the past. For example, *EcoK*, SP and SQ all share a common AAC domain while GAG is common to both *EcoB* and SB. One response of bacteriophages to the presence of restriction enzymes in their hosts is to mutate the recognition sites in the phage genomes, making them resistant to restriction^{13,14}. It is therefore advantageous for the host to be able to change the recognition specificity of its restriction system from time to time.

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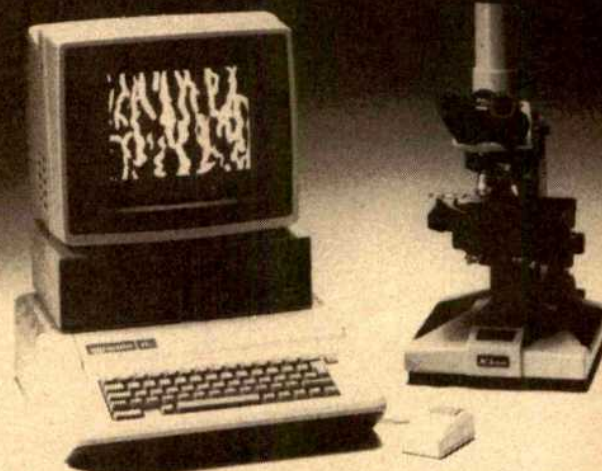
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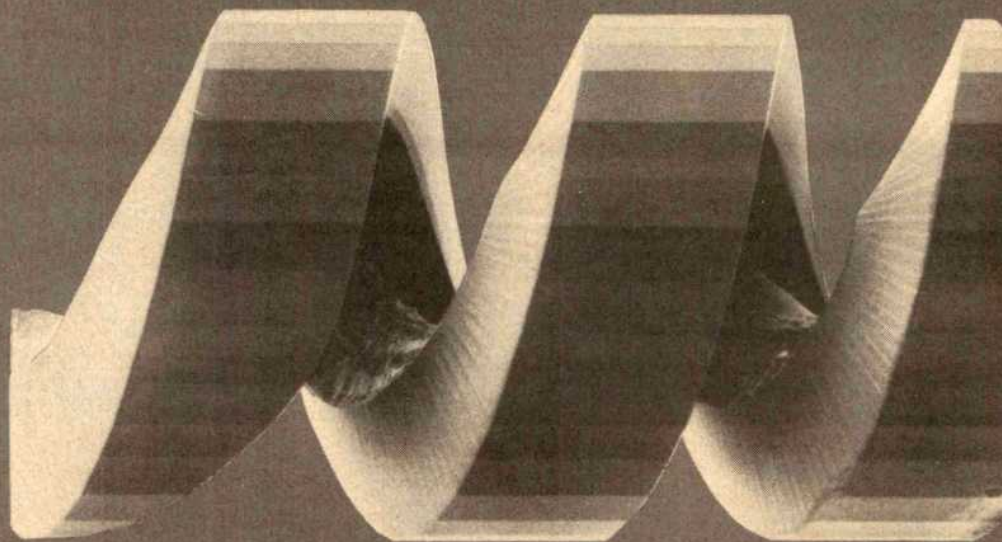
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The laser — hero or villain?

from Bridget Marx

Although too often associated in people's minds with star wars weaponry the laser has become an invaluable tool in many varied and peaceful applications.

IN the early days the laser, for Light Amplification by Stimulated Emission of Radiation, was dubbed 'a solution looking for a problem', but in the last quarter of a century lasers have found application in areas as diverse as materials processing and the treatment of cancer.

The medical application of lasers is now in fact a major industry—out of an estimated total commercial laser market of about \$400 million, at least one quarter is connected with medical and surgical applications. This market is growing at a rate of more than 30% per year and has become such a significant factor for the manufacturers of lasers that several companies have actually been bought out by medical conglomerates.

Laser surgery

The laser was first demonstrated in 1960 by Theodore Maiman at the Hughes Research Laboratories in California, and the first 'medical' application came when Maiman's ruby laser was being used in animals' eyes, as a source for photocoagulation and by 1962 human patients were being treated. As new lasers became available these were quickly tried out in the medical field. The argon ion laser was first considered in 1965, the clinical trials were started in 1969 and the first commercial systems became available in 1971. The argon laser has proved a lot more successful for the treatment of vascular diseases of the retina than the original ruby systems and there are now many thousands of medical argon lasers worldwide. At the time of writing lasers in routine use in hospitals include the argon ion laser, the carbon dioxide laser, the Nd:YAG laser and, to a lesser extent, the krypton ion laser. Those at present in the early stages of development include the excimer,

gold vapour and copper vapour lasers.

The laser is in many ways ideally suited to medical applications, providing as it does a light source that is powerful enough to destroy the target tissue yet is controllable in terms of power, spot size and wavelength. The light can be easily and efficiently taken to the required site either directly or by optical fibre. These advantages led to the rapid development of laser surgery in dermatology, ophthalmology and gynaecology. Other exciting areas are also opening up, such as photoradiation therapy for the treatment of cancer, laser recanalization of clogged arteries, laser welding of soft tissues, and even laser acupuncture for pain relief.

Diagnostics

The direct surgical use of lasers is not the only clinical application of lasers, and after photocoagulation, the 'surgical' application which accounts for 40% of all water-cooled lasers in use, the makers of cytofluorescence equipment are among the biggest consumers of water-cooled ion lasers, accounting for a further 14% of the market. Lasers allow biological specimens to be analysed for their cell content and the different cells to be sorted into different containers depending on their light scattering characteristics. This method of detecting cancer cells is very straightforward and cell-sorting equipment is to be found in many hospital laboratories.

Non-medical

The largest market for lasers is still in academic and industrial research and development: nearly \$100 million out of the total \$400 million. A traditional area of pure research is spectroscopy, and other growth areas are laser Doppler velocimetry and holography. Holography

these days is used not just for making pretty pictures, but also for making non-forgeable security passes and credit cards. And even spectroscopy has left the realms of pure research as we move into the age of laser isotope separation. (Laser isotope separation accounts for more than one eighth of the total CW dye laser market each year.) There is also a large part of the world's laser research effort concentrated in the field of optical-fibre communications, so that in the future telephone messages will not be relayed by an electrical signal along a wire but will be carried by a modulation of a laser beam that is being conducted along an optical fibre.

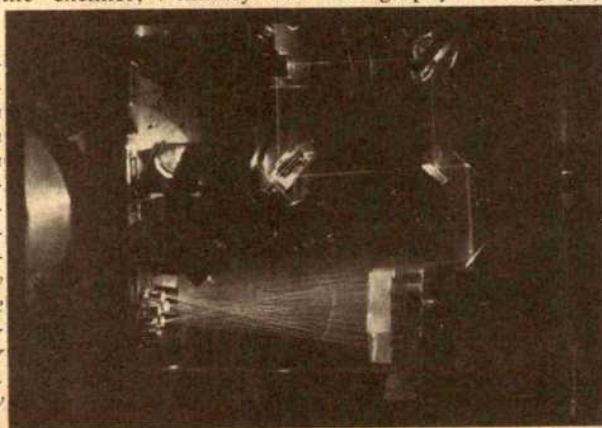
Popularization

Apart from these scientific applications there is a plethora of relatively 'low-tech' areas where lasers are carving out a niche. The entertainment industry is a market sector worth several millions of dollars each year. Laser light shows are quite commonplace these days in discotheques or at big public gatherings, as demonstrated during the 1984 Olympic Games in Los Angeles. There are also schemes using lasers to project television images onto a large screen with enough brilliance to be seen in daylight. And there are markets that use large volumes of low-cost lasers. In the entertainment industry the home video and audio disk systems come into this category. There are also laser printers and bar-code reading scanners at supermarket checkouts: these account for about 10% of the laser market, using approximately 100,000 small lasers a year.

In heavier industries too, lasers are gaining ground. Highpower infrared lasers are used for cutting, drilling and welding in laser machine tools. Ultraviolet excimer lasers are used in photolithography and other developmental techniques in the semiconductor industry.

So how will history judge the laser as it emerges from its formative years: Hero or villain? It has proved its worth in the areas of scientific research and manufacturing. It is capable of many medical feats, from miracle cure to routine surgery. President Reagan's Strategic Defense Initiative is based on the hope that it has an equally destructive side to its nature. □

The largest market area for lasers is still research and development. One application in which basic physics is tested is shown here. In this exotic application an argon ion laser is used to illuminate a long baseline interferometer for the detection of gravitational radiation. Interferometers with baselines of many kilometres are planned. The photograph shows an early prototype Michelson interferometer with a relatively short baseline. (Photo courtesy of Glasgow University.)



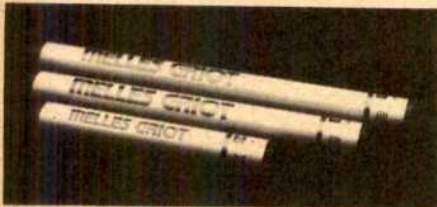
Bridget Marx is UK Sales Manager of the Laser Products Division of Coherent (UK) Ltd, Cambridge Science Park, Milton Road, Cambridge CB4 4BH, UK.

Lasers in commercial applications

A user-group based at Culham gives access to high-power lasers, and laser Raman microscopy is becoming established.

- A series of five reports from the Laser Analytics Division of Spectra-Physics should be of interest to all those using mass spectroscopy for the determination of trace moisture in hermetically-sealed integrated circuit packages. The reports show that the results of the infrared testing of IC packages sealed under controlled moisture conditions exhibit significant gaseous and absorbed moisture discrepancies when compared to less-accurate mass spectroscopy. Titles of the five are: (1) 'Water Vapor Measurements in IC Packages Using an IR Diode Laser'; (2) 'Trace Analysis of Moisture in IC Packages Using Derivative Diode Laser Spectroscopy'; (3) 'Laser Spectrometric Determination of Moisture in IC Packages'; (4) 'Dynamic Measurement of the Water Vapor Content of IC Packages Using Derivative IR Diode Laser Spectroscopy'; (5) 'Trace Analysis of Microvolume Gas Samples Using IR Spectroscopy Moisture in IC Packages'.

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The new Melles Griot laser is an alternative to solid-state systems.

- A new helium/neon laser with infrared output at $1.523 \mu\text{m}$ is now available from Melles Griot. The new laser matches the output of solid-state laser diodes, without the inherent problems, and should find applications in data transmission, intrusion alarms, target designation, range finding and security systems. Current models offer 0.25, 0.5 or 1.00 milliwatts of TEM₀₀ single longitudinal mode collimated coherent output. This output matches the minimum attenuation in the new high performance OVD fibres, falls in the middle of a clear atmospheric window, corresponds to the sensitivity peak of InGaAs detectors and is classified 'eye safe' by the US Bureau of Radiological Health. These $1.5 \mu\text{m}$ He/Ne lasers are constructed with zero-leak hardseals for longevity, and feature a patented symmetrical cold-cathode for stability. The instruments are based on the same design and manufacturing techniques as the company's established 632.8nm range.

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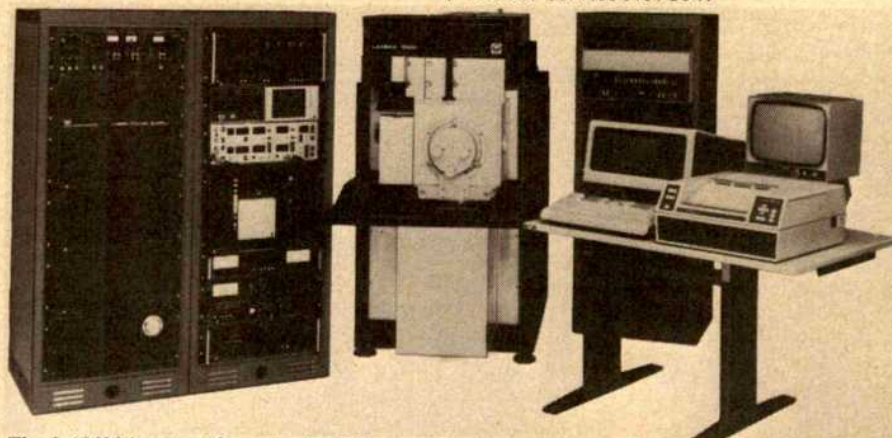
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- The Hybond Laser Spotlight Bonder, specially developed for wire-bonding, features enhanced accuracy provided by a laser spotlight which enables the work piece to be positioned quickly and with great precision. An integral 'soft-touch' ramping force generator protects the work piece against thermal shock. Precise control of position is achieved by a combination of easy-to-use adjustable controls for search, bond and loop height, with a motorized z function for true vertical movement. Quick visual reference and ease of fine adjustment is provided by the 'Paramagraph' controls. In the United Kingdom the bonder is available from Intertrade Scientific of Milton Keynes.

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- The Leybold Heraeus LAMMA 1000 uses a Nd:YAG laser to provide approximately 2mJ of light energy in 15 ns focused to $1 \mu\text{m}$ spot size. This laser power is used to ionize a micro-volume of the sample which is held on a precision x-y-z table in the sample chamber. The ions formed are then accelerated into a time of flight drift tube and are mass resolved. The ion detector at the end of the drift tube has a copper-beryllium dynode electron multiplier giving an amplification of better than 10^6 . The ease of putting a sample into the instrument with the minimum of sample preparation makes this instrument particularly useful for analysing small bulk samples. The lateral resolution allows the determination of distribution of atomic or molecular species. It is also possible to trace stable isotopes and to do depth profiling with LAMMA. By reducing the laser power, larger ionic fragments are produced for analysis of organic substances. It is possible to obtain positive or negative spectra and masses greater than 1,000 amu can be resolved. LAMMA can detect beryllium, lithium, helium and hydrogen.

Reader Service No. 103.



The LAMMA system from Leybold Heraeus resolves masses greater than 1,000 amu and easily detects beryllium, helium and hydrogen.

- Culham Laboratory's Laser Application Group produces two multi-kilowatt lasers, the CL5 and CL10, together with the associated beam-manipulating and work-handling facilities. Applications include material processing, such as cutting, welding and surface treatment, high power CO₂ laser beam propagation, monitoring and quality control. The Laser Applications Group, which is situated at the UK Atomic Energy Authority's Culham Laboratory, carries out contract research and development on high power laser systems for customers from both industry and government-funded organizations. The group has assembled an extensive range of CO₂ lasers, with output powers from a few watts up to greater than 10kW. A wide variety of beam-manipulating and work-piece handling equipment has also been developed and experience has been gained in the propagation of multi-kilowatt laser beams over distances greater than 50m. Reliable techniques have been developed for materials processing and for analysing the laser beam quality. This expertise enables the Laser Applications Group to offer customers services from a short assessment to the provision of a complete laser-based flexible manufacturing system. Development programmes are undertaken either for individual customers or on a group-sponsored basis. A 'Laser Users' Club' has been formed at Culham to provide the member companies with the necessary knowledge of laser processing technology for them to remain competitive. The Laser Club programme, has been sponsored jointly by eight companies and the Department of Trade and Industry. The Laser Applications Group aims to establish other club activities, particularly with European participation, and these could be directed towards specific industries.

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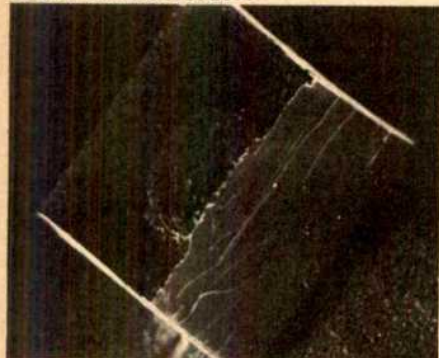
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• New from Labsphere is an infrared integrating sphere for CO₂ laser power measurements which uses a 4-inch diameter water-cooled sphere with diffuse gold coating. The WIS-4000 can be used in conjunction with almost any infrared detector which mounts at a port on the integrating sphere. The high power levels of infrared lasers are attenuated to a 'safe' value within the operating range of the detector and alignment sensitive measurement errors are eliminated. The WIS-4000 can also be mounted at the back mirror of the laser cavity to monitor actual power levels during operation. The WIS-4000 is normally supplied with a gold coating optimized for diffuse reflectance at the CO₂ laser wavelength. However, the sphere may be supplied optimized at other infrared laser lines as well. Accessories for the WIS-4000 include a water-cooled laser target used to reflect the first strike of the laser and a constant temperature recirculating cooler.

Reader Service No. 109.



New from Spectra Physics, a single-mode tunable diode laser.

• The new range of short-cavity lead-suit lasers from the Laser Analytics Division of Spectra Physics provides substantially improved single-mode performance at liquid nitrogen temperature and above. The latest lasers make possible the achievement of single-mode, CW emission from short-cavity mesa-stripe geometry Pb-salt diode lasers in 5, 10 and 30 μm spectral regions. The new devices, with a cavity length of 120 μm exhibit a Fabry-Perot mode separation of 6 cm^{-1} . Exploiting the spectral narrowing of the optical gain characteristics with increasing operating temperature single-mode operation can be obtained over broad current ranges in the 5 and 10 μm spectral regions at liquid nitrogen temperature and above. For the 30 μm lasers, single-mode operation is possible in short-cavity lasers at current values of up to almost twice threshold. Single-mode tunable diode laser (TDL) operation in the mid-infrared spectral region is of great interest in applications such as high-resolution infrared spectroscopy, heterodyne detection, and, in the future, mid-infrared fibre transmission. In addition, operating such lasers at liquid nitrogen temperature and above greatly simplifies the cooling requirements.

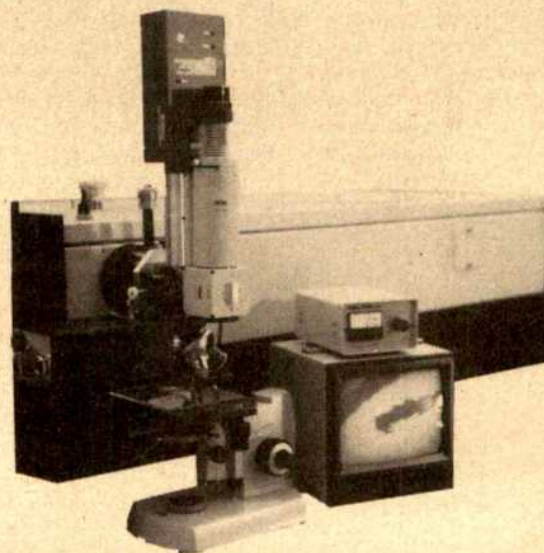
Reader Service No. 110.

• A series of Laser Beam Analysers (LBA) manufactured in West Germany by ALL is now available in the United Kingdom. The LBA accurately monitors the spatial distribution of a laser beam intensity in two orthogonal axes. The instruments use a highly reflective molybdenum needle rotating at 50Hz perpendicular to the beam path that reflects a fraction of the beam onto two pyroelectric detectors. The amplitude of the signals from these detectors displayed simultaneously on a dual trace oscilloscope corresponds to the power distribution through the beam. The LBA can handle beam powers from 10W to 20KW, with power densities up to 10 W cm^{-2} . As the power loss of the beam is only 0.8% as it passes through the LBA, the instruments can be used for on-line monitoring of beam profiles during experiments and material processing. The LBA can monitor changes in the mode before they become apparent in the quality of the laser processing. Other applications include measurement of beam divergence and beam focus by movement along the laser beam path.

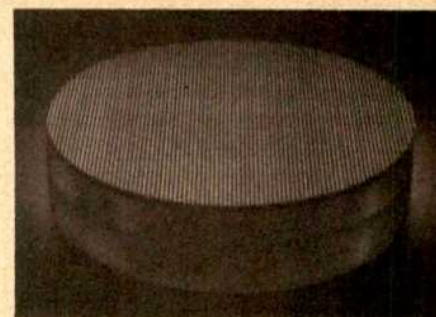
Reader Service No. 111.

• Laser Raman microscopy is now established as a powerful analytical technique. It provides a means of examining and chemically analysing samples as small as 2 μm in size in a non destructive way. The laser beam, often with a power of less than 100 mW, enters the microscope and is focused on the area of the sample under investigation. The Raman scattered light is collected and enters the spectrometer which then produces the vibrational spectrum of the sample. The technique has proved invaluable for studying optical fibres, semiconductor films, lubricants, corrosion effects, fluid inclusions in geological samples, biological tissues, waste products and polymers. Application notes are available on most of these applications from Glen Creston Instruments.

Reader Service No. 112.



Glen Creston Instruments' laser Raman microscope can analyse samples only 2 μm in size in a non-destructive manner. Applications include study of fluid inclusions in geological samples as well as analysis of biological tissues.



The Littrow-mounted diffraction grating end reflector.

• The Optometrics Group, long established in the field of photonics, has introduced the model LA-1000, the latest in a series of light harnessing instruments. This infrared Laser Attenuator gives researchers greater flexibility in experiments requiring precision attenuation of infrared laser beams. The LA-1000 is primarily designed for linearly polarized lasers, especially CO₂ lasers at 10.6 μm . For unpolarized lasers, it is possible to use two attenuators in series. Also from Optometrics is a new system for wavelength tuning and selection. The output wavelength of a laser can be tuned by turning the Littrow-mounted diffraction grating end reflector about an axis parallel to the grooves. The equation relating wavelength to angle of incidence on the grating is $n\lambda = 2d \sin i$. Using this relationship, one can calibrate a mirror mount to i using a grating as one of the reflectors in the laser cavity. This will narrow the spectral line width of the output in a region around the Littrow wavelength.

Reader Service No. 113.

• Spectra Diode Labs has introduced a new range of 200-mW continuous wave diodes. Claimed to be the most powerful diode lasers commercially available, they should find a niche in such fields as solid-state lasers, and fibre optic power delivery.

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Wildlife Pest Control Around Gardens and Homes. By TERRELL P. SALMON and ROBERT E. LICKLITER. University of California Division of Agriculture and Natural Resources. 1984. Pp.90. Pbk ISBN 0-931876-66-4. Np.

Archaeology

Excavations at Grimes Graves Norfolk 1972-1976. By JULIET CLUTTON-BROCK. British Museum Publications. 1985. Pp.46. Pbk ISBN 0-7141-1374-3. Pbk £10.

The Greek Trireme of the 5th Century B.C. JOHN COATES and SEAN McGRAIL (eds). National Maritime Museum, Greenwich. 1985. Pp.140. Pbk ISBN 0-905555-95-3. Pbk £8.

Paleopathology at the Origins of Agriculture. MARK NATHAN COHEN and GEORGE J. ARMELAGOS (eds). Academic. 1984. Pp.615. ISBN 0-12-179080-0. \$59, £41.50.

The Palynology of Archaeological Sites. By GEOFFREY W. DIMBLEBY. Academic. 1985. Pp.176. ISBN 0-12-216480-6. \$45, £46.

General

Handbook of Discourse Analysis, Vol.1. Disciplines of Discourse. TEUN A. VAN DIJK (ed.). Academic. 1985. Pp.302. ISBN 0-12-712001-7. \$59.50, £45.50.

High Altitude Deterioration. J. RIVOLIER et al. (eds). Karger. 1985. Pp.228. ISBN 3-8055-3972-X. DM 174, \$61.75.

Ideas, Qualities and Corpuscles: Locke and Boyle on the External World. By PETER ALEXANDER. Cambridge University Press. 1985. Pp.336. ISBN 0-521-26707-2. £27.50.

Image Understanding 1984. SHIMON and WHITMAN RICHARDS (eds). Ablex Publishing, 355 Chestnut Street, Norwood, New Jersey. 1985. Pp.268. ISBN 0-89391-254-9. \$35.

Modern Trends in Hypnosis. DAVID WAXMAN et al. (eds). Plenum. 1985. Pp.429. ISBN 0-306-41905-X. Np.

Multivariable Control: New Concepts and Tools. SPYROS G. TZAFESTAS (ed.). Reidel. 1984. Pp.502. ISBN 90-277-1829-6. Dfl 1230, \$49.50, £33.25.

Nuclear Fact Book, 2nd Edn. A.M. PLATT, J.V. ROBINSON and O.F. HILL (eds). Harwood Academic. 1985. Pp.176. ISBN 3-7186-0273-3. \$33.

On the Frontier: Flight Research at Dryden, 1946-1981. By RICHARD P. HALLION. NASA. 1984. Pp.385. Np.

Proceedings of the Royal Institution of Great Britain, Vol. 56. NINA HALL and IVOR WILLIAMS (eds). Science Reviews. 1984. Pp.288. ISBN 0-905927-81-8. Np.

Public Health Papers, Vol. 81: Community Response to Alcohol-related Problems: Review of an International Study. By E.B. RITSON. World Health Organisation. 1985. Pp.58. ISBN 92-4-130081-7. Np.

The Regional Economic Impact of Technological Change. A.T. THWAITES and R.P. OAKLEY (eds). Frances Pinter. 1985. Pp.249. ISBN 0-86187-373-4. £17.50.

Research: How to Plan, Speak and Write About it. CLIFFORD HAWKINS and MARCO SORGI (eds). Springer-Verlag. 1985. Pp.184. ISBN 0-387-13992-3/ ISBN 3-540-13992-3. DM 32.

Revelations: Glimpses of Reality. RONALD S. LELLO (ed.). Shephard-Walwyn. 1985. Pp.136. ISBN 0-85683-079-8. £8.50.

Safeguarding the Atom: A Critical Appraisal. JOZEF GOLDBLAT (ed.). SIPRI. 1985. Pp.243. ISBN 0-85066-306-7. Np.

Science and Technology Policies in Finland and Hungary: A Comparative Study. K.O. DONNER and L. PAL (eds). Akadémiai Kiadó, Budapest. 1985. Pp.371. ISBN 963-05-3977-2. £19.75.

United States Arctic Interests: The 1980s and 1990s. WILLIAM E. WESTERMEYER and KURT M. SHUSTERICH (eds). Springer-Verlag. 1984. Pp.369. ISBN 0-387-96009-0. DM 114.

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NOTTINGHAM UNIVERSITY

DEPARTMENT OF BIOCHEMISTRY

POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for a post-doctoral position to isolate and characterise gene sequences for proteins associated with specific brain diseases. The work will establish the origin and regulation of the proteins concerned, and will prove useful in the molecular diagnosis of the diseases.

This grade 1A Wellcome Trust-supported position, tenable for three years has a starting salary of 7,520 pounds (under review) including USS benefits.

Applicants with suitable experience in recombinant DNA technology should send a curriculum vitae and the names of two referees to Dr Robert E. Glass and Dr Michael A. Billett, Department of Biochemistry, Medical School, Queen's Medical Centre, Nottingham NG7 2UH (telephone 0602-700111) from whom further information may be obtained. (7193)A

THE LONDON HOSPITAL MEDICAL COLLEGE (University of London)

MLSO IN HISTOPATHOLOGY

Applications for a MLSO in Histopathology are required for the Experimental Pathology Department of the London Hospital Medical College. HNC/TEC pass or an appropriate science degree required, and facilities are available for taking further qualifications. The job entails the assistance with experiments, radioactive isotopes, upkeep and development of computer programs for record keeping, audio visual aids and image analysis. Post vacant with effect from 1st October 1985.

Salary range MLSO £6,488 to £8,789 inclusive of London Weighting (under review). Application forms are available from the Assistant Secretary, the London Hospital Medical College, Turner Street, London E1 2AD (01-377-8800 ext 12), and should be returned within 21 days of this advertisement. (7194)A

INSTITUTE OF DERMATOLOGY UNIVERSITY OF LONDON WELLCOME SKIN PHARMACOLOGY LABORATORIES

Postdoctoral research associate (pharmacology) required for MRC Programme Grant-supported research on molecular mechanisms of skin inflammation. The current emphasis of the work is on lipoxigenase products and studies are in progress in human subjects and using cultured keratinocytes. The successful candidate, who will be responsible to the Professor of Dermatology, should have substantial post-doctoral experience in pharmacological aspects of inflammation and will be considered for an honorary lectureship in the Institute. Salary scale: £7,520-£12,150 plus London Weighting.

Applications, with the names of two referees, to: Professor M.W. Greaves, Wellcome Skin Pharmacology Laboratories, Institute of Dermatology, Homerton Grove, London E9 6BX. (7192)

ROYAL HOLLOWAY AND BEDFORD COLLEGES

Egham Hill, Egham,
Surrey TW20 0EX

DEPARTMENT OF BIOCHEMISTRY RESEARCH ASSISTANT

Required for 3 years from 1st October 1985 to work with Dr. J. R. Bowyer on the electron acceptors of chloroplast Photosystem II. Candidates should have a least a 2(i) Honours Degree in Physics or related science with a knowledge of electronics and optical instrumentation, and a desire to apply this knowledge to a biological system. The project involves the construction of a very sensitive rapidly-responding spectrophotometer and its application to measure absorption changes associated with photosynthetic electron transport. Candidates may register for a higher degree. Salary will be on the 1B range for Research Staff on a scale £7,833-£8,753 inclusive of London allowance.

Applications with cv and the names and addresses of 2 referees should be sent to Mrs D. J. Odds, Personnel Officer. (7200)A

OFFICE OF INTERNATIONAL AFFAIRS OFFICE OF THE DIRECTOR NATIONAL CANCER INSTITUTE PUBLIC HEALTH SERVICE DEPARTMENT OF HEALTH AND HUMAN SERVICES

ASSOCIATE DIRECTOR FOR INTERNATIONAL AFFAIRS

The National Cancer Institute (NCI) interacts with scientists throughout the world to promote the advancement of cancer research, treatment and prevention. These activities result in bilateral agreements with other countries for cooperative research, the collection and dissemination of scientific knowledge worldwide, and the increased use of computer technology to improve these information transfers. The Office of International Affairs (OIA) is responsible for the planning, coordination, and management of these efforts within the NCI. OIA has an annual operating budget of approximately \$9 million.

This is a Civil Service position in the Senior Executive Service (SES) with a salary range from \$61,296 to \$68,700 per annum. Candidates may be eligible for Physicians' Comparability Allowance up to \$10,000 per year. (Alternately, candidates may be eligible for appointment in the Commissioned Corps of the U.S. Public Health Service.) The individual selected, if not presently in SES, must serve a one year probationary period.

Candidates must meet established mandatory professional/technical and managerial/executive requirements. Candidates will be further evaluated on the extent they exceed the minimum requirements and on additional factors including scientific reputation/peer recognition, training and education, and honors and awards. A copy of the requirements may be obtained by contacting **Ms. Valerie Barbour, Personnel Management Branch, NCI, (301) 496-6864.**

Applications (Personal Qualifications Statement SF-171) are to be sent to the above individual at: **The National Cancer Institute, Building 31, Room 3A-23, 9000 Rockville Pike, Bethesda, Maryland 20205.** A current curriculum vitae and bibliography must accompany all applications. Applications from women and minority group members are especially solicited.

*The National Institutes of Health is an
EQUAL OPPORTUNITY EMPLOYER.*

(NW55)A

Experimental Solid State Physicists

Vacancies exist for solid state physicists to work in the neutron scattering experimental programme at the Rutherford Appleton Laboratory's Spallation Neutron Source. The SNS is an intense, pulsed source which has recently become operational and which is of world significance to future research in the fields of physics, chemistry, biology and materials science.

The appointees would be expected to advance the use of neutron inelastic scattering in the study of condensed matter and the duties would involve developing new neutron spectrometers as well as providing experimental support to University groups which use the SNS facility. Candidates are also expected to be capable of making significant contributions of their own to the future SNS scientific programme. Previous experience in neutron, x-ray or electron scattering techniques is desirable but not essential. Applicants should however have demonstrated their research potential and ideally have some experience of instrumentation development or computing.

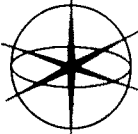
The appointments will be made in the Higher Scientific Officer grade salary range £7788-£10541 per annum.

Candidates should have a degree or equivalent in a scientific subject, plus at least 2 years post graduate research experience for applicants with 1st or 2nd class honours degrees or their equivalent. Other applicants are required to have at least 5 years of appropriate scientific experience after qualifying.

The Rutherford Appleton Laboratory is a friendly community with its own restaurant and sports facilities. Our transport covers towns and villages in the area. Excellent working conditions and generous holidays apply. This is a pensionable appointment.

Contact Recruitment Office, Personnel Group, Science and Engineering Research Council, Rutherford Appleton Laboratory, Chilton, Didcot, Oxon OX11 0QX, Tel: (0235) 445435, quoting reference VN 364.

Closing date: 16th August 1985

serc  **Rutherford Appleton Laboratory**
(7201)A

pathologist drug safety evaluation

An opportunity exists in the Pathology Group at SEARLE RESEARCH and DEVELOPMENT Center located in the South of France.

The candidate should have a D.V.M., M.D. or Ph.D degree in Pathology and at least five years experience (preferably in the pharmaceutical industry) in veterinary pathology.

The successful candidate will act as pathologist (slide reading and interpretation) on preclinical safety studies and will be involved in pathology research programs.

SEARLE R & D offers excellent laboratory facilities; competitive salaries, benefits and paid relocation. The Center is located in a particularly attractive part of France between Nice and Cannes, and is a part of G.D. SEARLE and Co., an international pharmaceutical company.

Applications with a full C.V. including current salary should be addressed to :

Mr G. du Parc, Personnel Manager,
SEARLE Recherche et Développement
Sophia Antipolis - B P. 23
06561 VALBONNE Cedex, FRANCE. (Ref. N.7)

SEARLE

(W2049)A

THE AUSTRALIAN NATIONAL UNIVERSITY RESEARCH SCHOOL OF EARTH SCIENCES POSTDOCTORAL FELLOW/ RESEARCH FELLOW/ SENIOR RESEARCH FELLOW IN ORE-GENESIS STUDIES

Applications are invited from suitably qualified women and men for appointment as Postdoctoral Fellow Grade 1, Research Fellow or Senior Research Fellow in ore-genesis studies in the Research School of Earth Sciences. The appointee will work with Dr I. H. Campbell, Senior Fellow, in developing research in this field. The School wishes to make the appointment at the Research Fellow level, but will be prepared to consider applications at the level PDF or SRF. The appointment initially will be in the Geophysical Fluid Dynamics Group of the School, but it is possible that the ore-genesis work will be organized as a separate research group as it becomes well established.

The overall brief for ore-genesis studies is to carry out fundamental research, identify fundamental problems which are of interest to other groups within the School, and coordinate the School's total programme in ore-genesis studies. Collaboration with relevant organizations outside the School, including Australian mining companies, will be important.

Persons with suitable backgrounds in geology and geochemistry are encouraged to apply.

Closing date: 13 September 1985.
Ref: ES27.6.1.

Conditions of appointment: Salary will be in accordance with qualifications and experience within the range: Postdoctoral Fellow Grade 1 \$A22,614-\$25,905 p.a.; Research Fellow \$A26,236-\$34,467 p.a.; Senior Research Fellow \$A36,649-\$43,715 p.a.; current exchange rate \$A1=UK 51p.

Appointment, unless otherwise stated, will be: Senior Research Fellow/Research Fellow normally up to three years with the possibility of extension to maximum of five years; Postdoctoral Fellow normally two years, with the possibility of extension to maximum of three years.

Grants are provided towards travel and removal. Assistance towards housing is given to an appointee from outside Canberra. Eligible appointees will be required to join the Superannuation Scheme for Australian Universities. Maternity leave is available. The University reserves the right not to make an appointment or to make an appointment by invitation at any time.

Applications should be submitted to the Acting Registrar, Australian National University, GPO Box 4, Canberra, ACT 2601, Australia. Applicants should quote the reference number when submitting applications and when requesting further particulars, which are also available from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

The University is an Equal Opportunity Employer. (W2050)A

ETH ZÜRICH

The Swiss Federal Institute of Technology in Zurich invites applications for a faculty position in Food and Agricultural Chemistry.

Duties of the new professor include the teaching of food chemistry and food biochemistry (including laboratories) for food science students and, in cooperation with other professors, organic chemistry for students in agriculture and forestry.

The successful candidate will have proven ability to conduct research in the chemistry, biochemistry and analysis of foodstuffs and talent for teaching at all university levels.

Applications with c.v. and list of publications should be submitted before September 30, 1985, to the President of ETHZ, Prof. H. Ursprung, ETH-Zentrum, 8092 Zurich, Switzerland. (W2046)A

Senior Chemist for Peptide Synthesis

To strengthen our developing medicinal chemistry group, we are looking to appoint a chemist who will play a significant role in the synthesis of novel peptides.

We are Merck Sharp and Dohme Research Laboratories, Neuroscience Research Centre, newly established on a 30 acre site near Harlow, Essex. Initially focussing on the study of inhibitory chemical messengers in the brain and exploration of the functional role of neuropeptides, we shall be one of the world's largest centres dedicated to neuroscience research.

Applications are invited from candidates who are Ph.D. Chemists with a minimum of two years postdoctoral experience and have specialised in peptide synthesis and are seeking a permanent post in medicinal chemistry research. Alternatively, consideration will be given to outstanding Ph.D.'s who have specialised in peptide synthesis for a postdoctoral fellowship of one to two years duration. Applications enclosing a full C.V. to:

Mr. P. A. Harley, Personnel Manager, Merck Sharp and Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Eastwick Road, Harlow, Essex CM20 2QR.



MERCK
SHARP
DOHME



Neuroscience Research Centre

(7221)A

BIOCHEMISTRY

Microgenics is a biotechnology company which has developed an advanced and proprietary diagnostic test system based on protein engineering by recombinant DNA techniques. New positions are available for exceptional research scientists.

IMMUNOCHEMISTS, PhD. Candidates will supervise product development of CEDIA homogeneous enzyme immunoassay. A PhD in immunology or chemistry and prior industrial product development experience required. Candidates will be familiar with validation of diagnostic assays, generation of GLP/GMP protocols, and submission of FDA documentation.

CHEMISTS/BIOLOGISTS, BA/BS/MS. Candidates will be familiar with the performance and documentation of an EIA/ELISA-510(k) validation. Responsibilities will include development of commercial immunoassays and applicable FDA documentation. Supervisory experience preferred.

MOLECULAR BIOLOGISTS, PhD. Candidates will protein engineer hybrid and tribrid polypeptides at the recombinant DNA level for commercial immunoassays within eukaryotic and prokaryotic expression vectors.

CHEMISTS/BIOLOGISTS, BA/BS/MS. Candidates will function in protein chemistry with a practical knowledge of liquid chromatography, SDS-PAGE, Western-Blots and enzyme-immunoassays.

Microgenics offers a scientific challenge and equity participation with competitive salaries and fringe benefits. For immediate consideration, forward your resume in strict confidence to:

MICROGENICS CORPORATION
2341 Stanwell Dr. • Concord, CA 94520



MICROGENICS

(NW71)A

CSIRO

AUSTRALIA

RESEARCH IN RESOURCE INFORMATION TECHNOLOGY

\$A26,001 — \$A38,243

DIVISION OF WATER AND LAND RESOURCES

CANBERRA ACT

CSIRO conducts scientific and technological research in laboratories located throughout Australia and employs about 7,500 staff, of whom some 2,900 are professional scientists. The Organization's research activities are grouped into five Institutes: Animal and Food Sciences, Biological Resources, Energy and Earth Resources, Industrial Technology and Physical Sciences. The CSIRO Division of Water and Land Resources is a member of the Institute of Biological Resources.

GENERAL: The Division of Water and Land Resources conducts research into methods for improved inventory, evaluation planning and management of the nation's water and land resources. The development of computer based methods for acquiring, storing, retrieving and analysing resource data for re-establishment of national, State and regional geographic information systems forms an important aspects of this research.

DUTIES. Collaborate in the research of the Catchment Hydrology Program to develop methods for the representation and analysis of spatially distributed data and the interpolation of spatial and temporal data through the definition of mathematical models for terrain and climate analysis. This program has research teams addressing questions in the fields of land and water resource management and in applications to catchment hydrology.

QUALIFICATIONS: A PhD degree or equivalent qualifications in mathematics and statistics and qualifications and/or experience in numerical analysis and computer programming, preferably including experience in the design and development of geographic information systems and in digital elevation modelling. Experience with processing and analysing cartographic data is desirable.

TENURE: Indefinite with Australian Government superannuation benefits available.

APPLICATIONS: Stating relevant personal particulars, including details of qualifications and experience, the names of at least two professional referees and quoting reference No A3132, should be directed to:

The Chief
CSIRO Division of Water and Land Resources
GPO Box 1666
CANBERRA ACT 2601
by 4 weeks from publication

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER (W2058)A

KEY CENTRE IN STATISTICAL SCIENCES LECTURESHIPS (LIMITED TENURE)

Applications are invited for several positions in the recently created Commonwealth Tertiary Education Commission (CTEC) Key Centre for Teaching and Research in the Statistical Sciences. The Key Centre involves the Departments of Statistics of La Trobe University and The University of Melbourne, the Statistics Section of the Department of Mathematics of Monash University and the Statistics and Operations Research Group of the Department of Mathematics, Royal Melbourne Institute of Technology. It is intended to make up to four appointments, initially for periods of up to three years. Some of the positions may involve statistical consulting as a major component of the duties. Appointees will be expected to have a strong background in some area of mathematical statistics or probability theory and to be active in research.

The positions are available immediately. Salary will be in the range \$A26,230–\$A34,467 per annum.

Further information concerning the positions for the Statistical Sciences Key Centre may be obtained from: The Director, Professor C. C. Heyde, Department of Statistics, The University of Melbourne, Parkville, Victoria 3052, Australia, telephone (03) 344 6609 or from the Heads of any of the constituent departments.

Applications, in duplicate, including the names and addresses of at least three referees should be addressed to: The Staff Officer, The University of Melbourne, at the above address, quoting position number 619 A575.

Applications close on 23rd September, 1985. Applicants should state whether they have a preference for appointment at one or other of the constituent institutions. (W2047)A

THE AUSTRALIAN NATIONAL UNIVERSITY

Applications are invited from suitably qualified men and women for appointment to the following positions:

John Curtin School of Medical Research

DEPARTMENT OF MEDICINE AND CLINICAL SCIENCE

POSTDOCTORAL FELLOW GRADE I (NBP)

The appointee will work with Dr. R. Stephens on the function and regulation of plasminogen activating enzymes in human cancer. Applicants should have a PhD degree, background in enzymology, cell biology, or tumour biology with biochemical perspective, and preferably experience in the generation and biochemical applications of monoclonal antibodies. Appointment will be for one year initially, with the possibility of extension for several months to a total period of about eighteen months. The position is funded through a National Biotechnology Program Research Grant Enquiries to Dr R. Stephens in the University (062) 84 2890. Background information may be obtained from the Acting Registrar.

Closing date: 22 August 1985. Ref. JC 6.6.1.

Faculty of Science

DEPARTMENT OF BIOCHEMISTRY

TEMPORARY LECTURER IN BIOCHEMISTRY (MICROBIOLOGY)

The successful applicant will be required to make a substantial contribution to the teaching of General Microbiology in a two semester course for second year science students. The research interests of the appointee should be compatible with one or more of the areas of microbiology established in the Department: molecular biology of antibiotic resistance and virulence in bacteria; alphavirus and flavivirus structure, replication and pathogenesis; cyanobacterial metabolism. The appointment is for 3 years from 1 January 1986 to 31 December 1988. Enquiries to Professor J. F. Williams in the University (062) 492284 or Dr P. R. Stewart (062) 492226.

Prospective applicants should write for further particulars to the Acting Registrar of the University.

Closing date: 16 August 1985. Ref: FS 4.7.1.

Conditions of appointment: Salary will be in accordance with qualifications and experience within the range: Postdoctoral Fellow Grade 1 \$A22,614–\$25,905 p.a.; Temporary Lecturer \$A26,236–\$34,467 p.a.; current exchange rate \$A1=UK50p.

Grants are provided towards travel and removal, though special conditions may apply to persons appointed to externally funded positions. Assistance towards housing is given to an appointee from outside Canberra. Eligible appointees will be required to join the Superannuation Scheme for Australian Universities. Maternity leave is available. The University reserves the right not to make an appointment or to make an appointment by invitation at any time. Applicants should quote the advertisement reference when submitting applications, and when requesting further particulars, which are also available from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Applications should be submitted to the Acting Registrar, Australian National University, GPO Box 4, Canberra, ACT 2601, Australia.

THE UNIVERSITY IS AN EQUAL OPPORTUNITY EMPLOYER. (W2055)A

TRENT POLYTECHNIC POSTDOCTORAL RESEARCH FELLOWS (TWO POSTS)

(Commencing Salary £8,010 — pay award pending)

DEPARTMENT OF LIFE SCIENCES

HERBICIDES

Required to join the Herbicide Research Group for a project entitled "The Physiology and Biochemistry of Herbicide Action". Applicants should have expertise in Plant Physiology/Biochemistry and be skilled in a range of biomedical techniques. Previous experience with herbicides and macromolecular synthesis would be advantageous but not essential. Ref NAB/LS/1.

BIOCHEMICAL PHARMACOLOGY

Required to join a small group investigating the importance of Ca^{2+} -mediated events to membrane interactions involved in stimulus secretion coupling in pancreatic islets and other tissues. Candidates should have a sound background in cell biochemistry with expertise in related techniques. Ref NAB/LS/2.

Please quote the reference number of the post for which you wish to apply. Further details and forms of application are available from the Staff Officer, Trent Polytechnic, Burton Street, Nottingham NG1 4BU. Closing date: 9th August 1985. (7208)A



Challenging Opportunities in Diagnostics R & D

Beckenham, Kent

The Wellcome Foundation Ltd. is an international group of chemical and pharmaceutical companies, with headquarters in the UK, and turnover close to £800m. Wellcome Diagnostics is an expanding commercial division of the group concerned with the design, development and manufacture of

diagnostic reagents.

At Wellcome's main research centre at Beckenham, we have opportunities for experienced scientists to take up key positions in the multi-disciplinary Department of Diagnostics R & D, a constituent of the Biomedical Research Division.

Section Leader (Virology) Starting salary up to £20,000

Leading a team of 11 post-doctoral scientists and graduate level technicians, you will contribute to the formulation and implementation of our research and development effort in this area.

You will possess at least 10 years' post-doctoral research experience with an acknowledged record of achievement in a virological field and preferably some knowledge of diagnostics. A flair for team leadership, the ability to respond dynamically to pressure and to interact effectively and authoritatively with commercial departments are essential attributes. Ref. WRL/351.

Research Virologist In excess of £12,000

To bring a successful background of virological research to our viral diagnostics team and the ability to make a major contribution to novel work in this area. Your post-doctoral experience of at least 5 years will have demonstrated a flair for creativity and innovation.

An ability to interact with both scientists and commercial personnel is essential. Ref. WRL/352.

Development Scientist (Bacteriology) In excess of £12,000

To lead a group of experienced bacteriologists involved in the development stages of the R & D process.

You will possess at least 5 years' post-doctoral bacteriology experience in an academic, industrial or hospital environment. Your function will include the provision of assistance to the Development Manager in the co-ordination and administration of development projects. Organisational skills and a flair for motivating scientific teams are essential, together with an ability to interact with research, production and marketing personnel. An aptitude for producing written communications is an essential requisite for this position. Ref. WRL/353.

Our starting salaries will reflect the importance of these positions and will be dependent on experience. Excellent benefits package includes five weeks' holiday, pension fund, sick pay scheme, subsidised restaurant and assistance with relocation expenses where appropriate.

Please write with full details, quoting the appropriate reference, to Dr. H. D. Avshu, Personnel Officer, The Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS.

(7226)A



Wellcome

BIOLOGIST

The Department of Molecular, Cellular, and Developmental Biology of the University of Colorado is seeking an Assistant Professor. The candidate's research specialty may be in any area where molecular techniques are now predominant: Plant biology, virology, developmental genetics, mechanisms of prokaryotic and eukaryotic gene expression, and molecular pathogenesis are some suitable areas. Evidence of ability to initiate and sustain an outstanding research program will be paramount, and the candidate should have postdoctoral experience. This tenure-track position will be available in September, 1986, though applications for the year following are encouraged.

Please send curriculum vitae, reprints, the names of three professional references, and other relevant material to: Search Committee, MCDB, Campus Box 347, University of Colorado, Boulder, CO 80309.

Deadline for application is October 15, 1985. Applications from women and members of minorities are sought and will be welcomed. (NW69)A

POSTDOCTORAL POSITION CARDIAC/SMOOTH MUSCLE ELECTROPHYSIOLOGY

Unique industrial and academic postdoctoral position to study ionic channel properties of isolated cardiac and smooth muscle cells and modulation by pharmacological and biochemical agents. Project will involve use of single cardiac and vascular smooth muscle cells for voltage clamp and internal perfusion as well as patch clamp techniques. Candidates should have a strong background in electrophysiology. For the first year, the successful candidate will work with: **Dr. J.R. Hume, Department of Pharmacology and Toxicology, Michigan State University, East Lansing, Mich. 48824;** the second year will be spent with: **Dr. Kai S. Lee at the Upjohn Company in Kalamazoo, Mich. 49001.**

Salary commensurate with experience and qualifications.

Send curriculum vitae and three letters of reference to: Dr. Hume (above address). Deadline for receipt of applications is September 1, 1985. (NW56)A

CSIRO

AUSTRALIA

REGIONAL

OFFICER-IN-CHARGE

\$A39,692 — \$A52,232

DIVISION OF SOILS

ADELAIDE LABORATORY

URRBRAE SA

CSIRO conducts scientific and technological research in laboratories located throughout Australia and employs about 7,500 staff, of whom some 2,900 are professional scientists. The Organization's research activities are grouped into five Institutes: Animal and Food Sciences, Biological Resources, Energy and Earth Resources, Industrial Technology and Physical Sciences. The CSIRO Division of Soils is a member of the Institute of Biological Resources.

GENERAL: CSIRO Division of Soils studies the biology, chemistry, mineralogy and physics of soils, as well as the integrative disciplines of pedology and geomorphology. It also seeks to apply the principles of soil science in agriculture, forestry, hydrology, engineering and conservation. The Division has laboratories in Adelaide, Brisbane, Canberra and Townsville with scientific management based on five research coordinators responsible to the Chief for the scientific direction and quality of research in the five major sub-disciplines of the subject.

The Division seeks an Officer-in-Charge of its Adelaide laboratories. These laboratories represent about half the complement of the Division, with a total staff of approximately 100, half of whom are professional scientists. **RESPONSIBILITIES:** The appointee, who will be an outstanding scientist, will provide scientific and administrative leadership for this laboratory, and will be directly responsible to the Chief of the Division, who is located in Canberra. He or she will be encouraged to rationally delegate responsibility, in order to maintain a strong personal commitment to scientific study and research. As principal representative of the Division in South Australia, the Officer-in-Charge will be expected to interact with State instrumentalities, the universities and other agencies with interests common to the Division of Soils.

The appointee will also be responsible to the Chief of Division of financial resource allocation in Adelaide. In the scientific stream this allocation will be effected in consultation with the five Research Coordinators; in the administrative area the site will be managed through professional administrative staff headed by a Divisional Administrative Officer responsible to the Officer-in-Charge.

QUALIFICATIONS: A PhD degree or equivalent qualifications plus an outstanding record of personal research achievement and leadership in a relevant field of soil science is essential. Broad management experience would be an advantage.

TENURE: Indefinite with Australian Government superannuation benefits available.

The appointment as Officer-in-Charge will be for an initial term of four years, with the possibility of a further term if mutually desired.

NOTE: Consideration will be given to appointment of an outstanding applicant at a higher salary.

A Higher Duties Allowance may be appropriate for the duties associated with the Officer-in-Charge position.

The Chief of the Division of Soils, Dr D E Smiles (Telephone Canberra 062 464911), would be pleased to discuss the position in more detail with potential applicants.

APPLICATIONS: Stating relevant personal particulars, including details of qualifications and experience, the names of at least two professional referees and quoting reference No A5254, should be directed to:

The Chief
CSIRO Division of Soils
GPO Box 639
CANBERRA ACT 2601
by 4 weeks from publication

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER

(W2057)A

THE OHIO STATE UNIVERSITY

CHAIR IN SCIENTIFIC COMPUTATION

The Department of Mathematics has been awarded a Chair in Scientific Computation. Applications are invited from individuals with outstanding credentials in any area of applied mathematics whose research activities make significant use of high speed computers. The appointee will hold the academic rank of Professor of Mathematics and will be expected to provide the academic leadership in the developing field of scientific computation at Ohio State.

Individuals Interested in this position should contact:

Alan Woods, Chairman
Department of Mathematics
The Ohio State University
231 W. 18th Avenue
Columbus, Ohio 43210
Telephone: 614/422-7173

(NW60)A

BOURN HALL CLINIC

Bourn, Cambridge, CB3 7TR

Embryologist required for a full-time position, joining a team providing 7 day cover on a rota basis working on human in vitro fertilisation.

Previous experience with animal embryology would be helpful, although a recently qualified graduate in a relevant subject would be considered.

Opportunity for research.

Please send full C.V. together with names of 3 referees to Professor R. G. Edwards FRS, Scientific Director, by 31st August 1985. (7224)A

Faculty Position available, tenure-track, in molecular genetics, with a special interest in membranes. Applicants should have research background in recombinant DNA technology (preferably also in somatic cell genetics) and working knowledge of membrane proteins. M.D. and/or Ph.D. in appropriate field required. Successful candidate will hold tenure-track appointment (Assistant or Associate Professor, depending upon qualifications) in Department of Physiology and Molecular Biophysics at Baylor College of Medicine. Joint appointments in relevant academic departments also possible. Laboratory space and basic equipment provided and initial laboratory support available. Successful candidate will devote majority of his/her effort to establishing and maintaining an independent research program related to the main interests of the Department. The Department is building a strong program in membrane biophysics with expertise in electrophysiology, membrane biochemistry, reconstitution, and modelling. Send vita, a brief description of past and present research experience, and names of three or more references to:

Dr. A. M. Brown, Incoming Chairman,
Physiology and Molecular Biophysics Department



Baylor College of Medicine,
One Baylor Plaza,
Houston, Texas 77030.

An Equal Opportunity A/A Employer

(NW58)A

UNIVERSITY OF WASHINGTON POST DOCTORAL POSITION

Available immediately, to study regulation of transcriptions of HLA genes, and to clone genes coding for transactive factors regulating HLA transcription. Experience with recombinant methodology desirable. Send statement of experience and interests, CV, names of references to:

D. Pious, Departments of Pediatrics and Genetics, RD-20, University of Washington, Seattle, WA 98195.

An Equal Opportunity Employer.

(NW68)A

Post Doctoral Positions (2)

Available in September and December, 1985 to study the molecular basis of action of insulin and related growth factors that activate tyrosine kinases/receptors.

Individuals are sought with training in biochemistry or cell biology with interests in either protein chemistry or the cell biology of intracellular vesicular traffic.

Please send curriculum vitae and the names of three references to: Dr. Paul F. Pilch, Department of Biochemistry, Boston University School of Medicine, 80 East Concord St., Boston, MA 02118. EOE/m/f. (NW59)A

Cytologists

...make a career move into applications marketing
salary negotiable to £14,000 plus car

This is an excellent opportunity for young cytologists involved in particular in screening for cancer of the cervix through the cervical smear technique (use of PAP stain etc.), to make their first move into marketing with an established bio-medical instrumentation company. As a leader in the field, innovation and outstanding design has become a hallmark.

To launch a new image analysis product an Applications Specialist is required to investigate user requirements in the cytology market. Key responsibilities include advising on strategic aspects of marketing, the development of product documentation packages, carrying out machine demonstrations on customer sites, training

end-users and prospecting for machine sales.

Candidates, aged ideally 25 to 35 will have a sound theoretical and practical knowledge of cytology as practiced in the UK and European medical establishments. They will be qualified to HNC/Degree level in Biology, Genetics, Botany, or Zoology. Most importantly, they must feel comfortable with the man-machine interface and should be able to demonstrate clear interpersonal and persuasive skills

Salary is negotiable to £14,000 plus fully expensed company car. The location is North Cheshire.

Applicants should write with full c.v. quoting ref. AR/073 to: March Personnel Services, 33 King Street, Manchester M2 6AA.

MARCH

PERSONNEL SERVICES

POST-DOCTORAL FELLOW

There is an immediate vacancy for a post-doctoral fellow in the Division of Basic Medical Sciences in the Faculty of Medicine. The stipend will follow MRC guidelines and is funded for up to two years.

The successful applicant will study, using a variety of techniques, the role of properties and nature of calcium channels in vascular smooth muscle and the liver.

Candidates with an appropriate pharmacological background are invited to apply.

Send curriculum vitae and the names of three referees to:

Professor C. R. Triggie
Associate Dean, Basic Medical Sciences
Faculty of Medicine
Memorial University of Newfoundland
St John's, Newfoundland, Canada A1B 3X6
Tel: (709) 737-6897

(NW51)A

POST-DOCTORAL POSITION

available after 1 October

Cell biology-physiology-biochemistry laboratory studying isolated cardiac membrane channel proteins seeks

IMMUNOLOGIST OR PROTEIN BIOCHEMIST

with immunological experience for immunofluorescence and immunoelectron microscopic investigations. Applicant should have doctorate and experience with poly- and monoclonal antibody techniques.

Send curriculum vitae, description of research interests, references to: Dr. Ernest Page, Department of Medicine, BH Box 137, The University of Chicago, 5841 South Maryland Avenue, Chicago, Illinois 60637. Equal Opportunity/Affirmative Action Employer. (NW45)A

POSITION OPEN

RESEARCH ASSOCIATE POSITION

Position available at the Research Associate level to work on the purification of lymphokines which regulate a B lymphocyte growth and differentiation. Applicants should possess a Ph.D. in a relevant field and have two or more years of postdoctoral experience. Familiarity with tissue culture methodology and commensurate with experience.

Send resumé and three letters of reference to:

Dr Peter Isakson
Box 448, Department of Pharmacology
University of Virginia Medical School
Charlottesville, Virginia 22908.

An Affirmative Action/Equal Opportunity Employer (NW50)A

M.R.C. CLINICAL RESEARCH CENTRE
(NORTHWICK PARK HOSPITAL)
WATFORD ROAD, HARROW
MIDDLESEX HA1 3UJ



NON-CLINICAL SCIENTIST

Applications are invited from post-doctoral microbiologists for a 3-year short-term appointment to study the host-parasite relationships between mammalian cells and bacteria, viruses and mycoplasmas. The research programme will be concerned with identification of glycoprotein or glycolipid receptors for infective agents on the host-cell membranes.

The successful candidate will join the Applied Immunochimistry Research Group headed by Dr Ten Feizi from whom further information can be obtained (Tel: 01-864 5311 ext. 2352).

Salary will be within the range £10,153-£16,158 (inclusive of London Weighting) depending on experience. Application forms are obtainable from Miss B. A. Shaw, Personnel Section, quoting reference 108/1/3905.

Closing date for applications 15th August 1985.

(7206)A

**University of Colorado
Health Sciences Center
Department of Biochemistry,
Biophysics and Genetics**

**Assistant Professor,
Molecular Biology**

Applications are sought to fill a tenure-track teaching/ research position in molecular biology, to begin on or about January 1, 1986. Candidates should have significant post-doctoral experience and will be selected primarily on the basis of their general qualifications.

To apply: send a vita, list of publications and the names and addresses of at least three references by October 15 to: Committee for Molecular Biology, B-121, University of Colorado Health Sciences Center, 4200 East Ninth Avenue, Denver, CO 80262.

The University of Colorado is an Affirmative Action/ Equal Opportunity Employer.

(NW66)A

**Postdoctoral Position in Cell and
Molecular Biology and Pharmacology**

Postdoctoral research position available to study the cell and molecular biology and pharmacology of arterial pressure control mechanisms in a collaborative interdisciplinary environment. Interests include molecular mechanisms of neurotransmitter storage and release, presynaptic regulation, and molecular mechanisms of cell signalling at neurovascular synapses including peptides, transmitters and receptor transduction. Projects provide opportunity for training with integrated facilities of transmission and scanning electron microscopy, cell culture, hybridoma technology, receptor techniques and molecular biology. Salary open with opportunity to progress to assistant professorship.

Interested candidates should send curriculum vitae and a brief statement of research objectives to: **Dr Thomas E. Gaffney, Chairman, Department of Pharmacology, Medical University of South Carolina, 171 Ashley Avenue, Charleston, South Carolina, 29425.**

(NW54)A

ANIMAL DISEASES RESEARCH ASSOCIATION

**Moredun Research Institute
Higher Scientific Officer (Immunologist/Virologist)
3 year appointment**

A 3 year appointment is available for a scientist with a PhD in Virology or Immunology to join a project investigating persistent viral infections. The successful candidate must be familiar with virological techniques and have an interest in the immunopathology of host responses to infection. Experience in immunochemistry and/or monoclonal antibody technology would be an advantage.

Salary scale: The current salary scale appropriate to the grade is: £7,788 rising by annual increments to £10,541. A non-contributory superannuation scheme is in force.

This is an equal opportunities post.

Further particulars may be obtained from **The Secretary, Animal Diseases Research Association, 408 Gilmerton Road, Edinburgh.** Closing date for receipt of completed application 1 September 1985.

Please quote reference 224/85 in all communications.

(7209)A

**THE INSTITUTE OF
CANCER RESEARCH**

Sutton, Surrey

**SENIOR TECHNICIAN
N HISTOLOGY**

(28 hours per week)

required to work with Pathologists and Scientists on aspects of human tumour diagnosis and complications of bone marrow transplantations. Previous experience in immunohistology is desirable. The successful candidate will be expected to handle frozen, paraffin and resin embedded material and to play a role in developing new techniques including in situ nucleic acid hybridization.

There is a close interaction between the Division of Pathology of the I.C.R. and the Department of Histopathology of The Royal Marsden Hospital.

Salary scale: £6,534-£7,802 (including Outer London weighting).

For further details contact: Dr J Sloane, Consultant Histopathologist, 01-643 5174 ext 24.

Applicants are advised that in the majority of The Institute's premises, smoking is prohibited.

Applications, in duplicate, with the names and addresses of two referees to be sent to The Personnel Officer, The Institute of Cancer Research, 17A Onslow Gardens, London SW7 3AL, quoting reference number 301/B/90.

Closing date: 8th August 1985.
(7203)A

**THE ANIMAL VIRUS
RESEARCH INSTITUTE**

SCIENTIFIC OFFICER

Applications are invited for the post of Scientific Officer to work in a group investigating the molecular biology of African Swine Fever virus. This virus contains a complex double-stranded DNA genome. The project involves analysis of the structure and function of the viral genome, using recombinant DNA methods and associated techniques. Collaboration with other groups within the Institute studying the immunology and pathogenicity of the virus is involved. The successful candidate will have a First or upper second class honours degree in a biological science, experience in molecular biology techniques is desirable.

Salary scale: £6,190 — £8,561.

22 days annual holiday, non-contributory superannuation and sickness benefit schemes. A house may be available for a suitable, married applicant.

Applications to include curriculum vitae and the names of two referees to be sent as soon as possible to the Secretary, The Animal Virus Research Institute, Ash Road, Pirbright, Woking, Surrey GU24 0NF. Tel: 0483 232441.

The Animal Virus Research Institute is an Equal Opportunity Employer. (7156)A

**UNIVERSITY OF
LIVERPOOL**

DEPARTMENT OF ORGANIC
CHEMISTRY

**SENIOR RESEARCH
ASSISTANT**

Applications are invited for a Post-doctoral Senior Research Assistant to work with Dr R. Cosstick in collaboration with Professor W. Fuller (University of Keele) on the effect of nucleoside analogues on DNA structure. The research involves the synthesis of polynucleotides using chemical and enzymatic techniques. Structural analysis of the polynucleotides will be performed by X-ray fibre diffraction using the SERC Synchrotron Radiation Source.

The post is best suited to a chemist or biochemist with an interest in DNA structure. The appointment will be for up to three years beginning as soon as possible after 1st October 1985.

Initial salary will be either £7,520 or £7,980 per annum (under review).

Applications, together with the names of three referees, should be received not later than 15 August, 1985, by the Registrar, The University, P.O. Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. Quote Ref. RV/160/N. (7199)A

**POSTDOCTORAL
RESEARCH POSITION**

is available in Molecular Parasitology to study developmentally regulated proteins of the protozoan parasite Plasmodia. Strong background in recombinant DNA technology required. Starting salary \$20,000-\$25,000 based on experience. Position available now.

Applicants should send curriculum vitae and the names and addresses of three references to: Dr Donald F H Wallach, Director of Radiobiology Division, Tufts-New England Medical Center, 171 Harrison Avenue, Box 824, Boston, MA 02111. Tufts University is an Equal Opportunity/Affirmative Action Employer. (NW52)A

**THE LONDON HOSPITAL
MEDICAL COLLEGE
(University of London)**

**POST-DOCTORAL
RESEARCH FELLOW**

Post-Doctoral Research Fellow required for MRC Supported project, with Dr L Henderson, Psychology Department, Hatfield Polytechnic, on motor control in Parkinson's disease. The suitable candidate will probably have completed a PhD in experimental Psychology or a related field. The starting date is 1st October 1985. Salary within range £7,520 — £8,450 plus London Weighting of £1,233. Renewable for 3 years.

For further information and application to Dr C Kennard, Department of Neurology, The London Hospital, London E1 1BB (01-247-5454 ext 49). (7180)A

UNIVERSITY OF LONDON
British Postgraduate Medical
Federation Institute of
Orthopaedics
DEPARTMENT OF BIOMEDICAL
ENGINEERING
LECTURESHIP IN CELL
BIOLOGY

Applications are invited for the post of Lecturer in Cell Biology tenable from 1st October 1985. The successful candidate will have had post-doctoral research experience and be familiar with the use of scanning and transmission electron microscopy as applied to the physical and biological sciences. Practical experience in microanalysis, techniques used in the identification of metals in biological specimens is essential. The appointee will be expected to maintain and run an analytical electron microscopy suite which provides a central service facility for the Institute and outside users and co-ordinate an ongoing active research programme involving clinical practice and industry.

Salary commensurate with qualifications and experience on the Lecturer Scale — £7,520 — £14,925 per annum + London Weighting Allowance.

Application must be accompanied by a full curriculum vitae, statement of research interest, experience and the names of three referees. Forms from: The Secretary, Institute of Orthopaedics, (University of London) Brockley Hill, Stanmore, Middlesex HA7 4LP. 01-954-2300 Ext 779. Closing date for receipt of completed applications 20th August.

(7179)A

**IMPERIAL COLLEGE OF
SCIENCE AND
TECHNOLOGY**
LECTURESHIP OR SENIOR
LECTURESHIP IN
EXPERIMENTAL LASER
OPTICS

available in the Department of Physics. An established reputation in experimental laser physics, preferably in the area of ultra-fast phenomena, nonlinear optics, optoelectronics or optical information technology, is an essential prerequisite. Appointee will join the Laser Optics group and will be expected to pursue a vigorous experimental research programme, and to engage in postgraduate teaching for a Master's degree course in Applied Optics, in addition to general departmental teaching duties, research student supervision etc.

The appointment will date from 1 January 1986 (or as soon thereafter as possible). Salary according to age and experience on the Lecturer or Senior Lecturer scale (currently £7,520 to £14,135 to £17,705) plus London Allowance of £1,233.

Detailed applications, including CV, list of publications and the names of at least three referees should be sent to Professor T W B Kibble, FRs, The Blackett Laboratory, Imperial College, London SW7 2BZ by 20 September 1985.

(7180)A

UNIVERSITY OF WAIKATO
Hamilton, New Zealand
LECTURESHIP IN
BIOLOGICAL SCIENCES
(BOTANY)
A85/13

Applications are invited from suitably qualified persons for a lectureship in Botany within the Department of Biological Sciences. The Department has an establishment of 15 academic staff teaching general biology at first year and specialist courses in all major disciplines at second and subsequent years. Over 60 research students are currently enrolled for higher degrees in Biology (M.Sc. and D.Phil) with particularly active groups in animal physiology, microbiology, biochemistry, thermophilic enzymes, plant ecology and physiology and limnology.

The appointee will be required to assist in teaching general undergraduate plant biology as well as developing more advanced teaching in the area of his/her speciality.

Preference will be given to applicants who hold postgraduate qualifications and research experience in one of the following areas: plant breeding, quantitative plant biology and modelling of natural systems, or comparative plant morphology.

Prospects for collaborative research within the Department, and with the local Agriculture and Forestry research establishments, are excellent, and the successful applicant will be encouraged to develop research activities allied to these local strengths.

The current salary range for Lecturers is NZ\$23,622–NZ\$27,928 per annum.

Interested applicants should obtain further details from the Registrar, University of Waikato, Private Bag, Hamilton, New Zealand, or from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Equality of employment opportunity is University policy.

Applications close with the Registrar on 23 August 1985. (W2052)A

**ROYAL FREE HOSPITAL
SCHOOL OF MEDICINE**
(University of London)
Department of Neurological
Science
TECHNICIAN

required to work in an EM neuropathology laboratory. Applicants must have a minimum of 2 years experience in basic histological and/or cellular immunological techniques.

Salary on Whitley Council Scale £5,738 – £8,096 (under review) plus £1,042 London Weighting Allowance.

Further particulars and application forms are available from the School Office, RFHSM, Rowland Hill Street, London, NW3 2PF or telephone 01-794-0500 extn 4262. Please quote reference PKT/T. Closing date 9 August (7204)A

CSIRO
AUSTRALIA

CHIEF OF DIVISION

**DIVISION OF MINERAL PHYSICS
AND MINERALOGY
NORTH RYDE NSW**

Applications are invited for the position of Chief of the Division of Mineral Physics and Mineralogy. Candidates should have an established record of scientific achievement and leadership, and proven experience in the applications of research in the fields of mineral exploration (by geophysical or geochemical methods, including instrumentation) or other relevant scientific or industrial disciplines.

Mineral Physics and Mineralogy is one of the nine Divisions of the Institute of Energy and Earth Resources, CSIRO, which encompasses a broad spectrum of research activities and industrial applications in minerals, energy and water resources. Chiefs of Divisions are responsible to the Director of the Institute and are expected to contribute to the corporate planning and operation of the Institute.

The Division of Mineral Physics and Mineralogy is part of a major CSIRO centre at North Ryde, a suburb of Sydney, and has recently been reorganized by combination of the geophysics and geochemistry research groupings at that location. It has a total staff of approximately 102 of whom about 68 are professional graduates.

The work of the Division is closely linked to the needs of the Australian mineral exploration industry, including fossil fuels. Its research applies the principles and techniques of physics, geology, geochemistry, geobiology and mathematics to the identification and solution of problems in mineral exploration. Current research includes concepts, techniques and applications in airborne and satellite remote sensing, rock magnetism and electromagnetic techniques, hydrochemistry, geobiology, isotope geology, ore genesis and exploration geochemistry. The major proportion of the research is conducted in collaboration with Australian mining companies and commercialization of the Division's inventions and intellectual property is a feature of its operation.

The Division has substantial in-house computing and analytical equipment, which includes a rock magnetism laboratory, heavy ion accelerator facility, mass spectrometers and electron beam microprobe.

The Chief of the Division will be responsible for the leadership, management, integration and application of the research programs and will continue and further develop where practicable the collaboration with industry and other institutions. Management skills and organizational ability, particularly in internal and external communication and the motivation of staff at all levels, are required.

The salary for the position will be not less than \$57,307 per annum plus an expense of office allowance of \$1,355. For an exceptional candidate a salary up to \$64,143 plus allowance of \$1,900 per annum would be offered.

Appointment to CSIRO is for an indefinite period and carries Australian Government superannuation privileges, subject to normal conditions. The position of Chief of the Division is offered for a negotiable term of five to seven years, with subsequent options for a further term, if mutually desired, or for a senior position in the Organization.

The Executive of CSIRO has appointed a Search Committee to seek suitable candidates for this position. The Chairman of the Committee is Dr A. F. Reid, Director of the Institute of Energy and Earth Resources, who would be pleased to provide further information about the Division and discuss the position with potential applicants. He would also be pleased to receive advice concerning the appointment from people with a particular interest in it. Dr Reid may be contacted in Melbourne by telephone (03) 542-2432 or telex 35675.

Applications stating full personal and professional details, and names of at least three professional referees and quoting reference number A3917 should reach:

The Director
CSIRO Institute of Energy and Earth Resources
Private Bag 10
CLAYTON VIC 3158
by 30 September 1985

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER

(W2061)A

WESTERN AUSTRALIA
**RESEARCH OFFICER
 — SHEEP NUTRITION**

CONTRACT POSITION
 DEPARTMENT OF AGRICULTURE
POSITION NO. 006051

SALARY: AGRICULTURAL SCIENTIST — Level 1 \$A21,349–\$A28,306 per annum or Level 2 \$A29,921–\$A32,436 per annum.

VETERINARY SCIENTIST — Level 1 \$A23,564–\$A30,722 per annum or Level 2 \$A32,436–\$A35,183 per annum.

Salary dependent upon qualifications and experience.

QUALIFICATIONS AND EXPERIENCE: Bachelor of Science (Agriculture) or a degree in Veterinary Science or approved equivalent. At least 5 years' post graduate experience required for appointment to Level 2. Post graduate qualifications or equivalent through publication or research in equivalent through publication or research in scientific journals desirable. Research experience in the partitioning of nutrients between body tissues and/or nutrition-reproduction relationships desirable.

DUTIES: Undertake research into the influence or nutrition on the reproductive performance of ewes, with particular reference to ovulation rate.

CONDITIONS OF SERVICE: Appointee will be required to enter into a contract for 3 years. Four weeks' annual leave, cumulative sick leave entitlements and other general conditions applicable to Temporary Officers employed under the provisions of the Public Service Act, 1978.

TRANSPORT AND REMOVAL ASSISTANCE: The cost of economy class air fares incurred by the appointee, spouse and dependent children under the age of sixteen years (where applicable), plus a reasonable contribution towards the cost of transporting essential personal and household effects to Western Australia.

GENERAL: For further information contact:

Dr R. W. Kelly
 Principal Officer
 Sheep and Wool Branch
 Department of Agriculture
 Telephone: (09) 367 0111

APPLICATIONS: To state experience, citizenship, qualifications and experience, with the names of two referees to the:

Chairman
Public Service Board
 111 St George's Terrace
 PERTH WA 6000

Closing: 2.00 p.m. Thursday, August 15, 1985.

ALL PUBLIC SERVICE APPOINTMENTS AND PROMOTIONS
 ARE BASED EXCLUSIVELY ON MERIT TO ENSURE
 EQUAL OPPORTUNITY FOR ALL. (W2067)A

UNIVERSITY OF
 EDINBURGH
 FACULTY OF MEDICINE
CHAIR OF PATHOLOGY

Applications are invited for the Chair of Pathology, which will fall vacant following the retirement of Professor Sir Alastair Currie on 31st March 1986. The Professor of Pathology will be expected to be of high academic standing, to have a wide interest in General Pathology, to have a substantial record of achievement in research, and to provide academic leadership as Head of the Department.

The Department has responsibilities for undergraduate teaching to medical and dental students, and for postgraduate teaching in the Faculty of Medicine. There is an Honours B.Sc. course in Pathology. The Department also has major responsibilities for the provision of diagnostic services to the National Health Service.

The salary will be at the clinical professorial level (£26,080 p.a.) if the successful candidate holds a registrable medical qualification or, if the successful candidate does not possess such a qualification, at the pre-clinical professorial level (minimum £18,555 p.a.); these salaries are under review.

Further particulars may be obtained from the Personnel Office, University of Edinburgh, 63 South Bridge, Edinburgh EH1 1LS, with whom applications (20 copies), including curriculum vitae and the names and addresses of three referees, should be lodged not later than 1st November 1985. Overseas candidates need submit only one copy of the application.

Please quote reference no. 31/85
 (7213)A

UNIVERSITY OF YORK

**CANCER RESEARCH
 UNIT**

**Research
 Assistant**

Applications are invited for a three year post, funded by the Health and Safety Executive, to work on the mechanism of mutation induced by environmental pollutants. Techniques to be used will include nucleic acid sequencing and gene-cloning. The successful candidate will be able to register for a higher degree. Applicants should have either a biological or chemical background and possess at least an Upper Second Class Honours degree.

Salary within the range
 £7,520–£8,920 per annum
 (currently under review).

Three copies of applications, with full curriculum vitae and naming two referees, should be sent by 9 August 1985 to Registrar's Department (Appointments), University of York, Heslington, York YO1 5DD. Please quote reference number 11/6198a.
 (7214)A

THE NATURE
 CONSERVANCY COUNCIL

SCIENTIFIC OFFICER:
 seabird colony register

The Nature Conservancy Council wishes to appoint an Ornithologist based at Aberdeen for a period of two years from early Autumn 1985.

The duties of the successful candidate will be to collate existing data on seabird breeding sites and to identify areas where further survey is needed. The appointee will liaise closely with members of voluntary ornithological and conservation societies, particularly the Seabird Group, and should be capable of co-ordinating work by large numbers of people. The work will be desk-based for most of the year, but some field-work will be involved in the summer. The post will be supervised by the Ornithologist in the Chief Scientist Directorate at Peterborough.

Qualifications and experience: The successful candidate should have a degree of equivalent in a biological or related subject and have experience of the use of computers. Ability to write computer programs and familiarity with seabirds would be advantageous. Applicants should have a current driving licence.

Salary: Dependant on qualifications and relevant experience: £6,190 rising to £8,561.

For further details and an application form, please contact Mr S. Woodfield, Personnel 1, Nature Conservancy Council, Northminster House, Peterborough PE1 1UA, to whom completed application forms should be returned by 15 August 1985.

The Nature Conservancy Council is an equal opportunity employer.
 (7218)A

MONASH UNIVERSITY

Centre for Early Human
 Development
**RESEARCH
 ENDOCRINOLOGIST**
 Monash-IVF Programme

Duties include: (1) Supervision of routine IVF hormone assays, (2) Development of experimental techniques to expand the Endocrine research component of this IVF programme and (3) Liaison with clinical colleagues and motivation of junior staff within the IVF programme. Appointee would have basic endocrine research facilities and possible appointment as Research Fellow in the Centre for Early Human Development and Department of Obstetrics and Gynaecology. Applicants must have a PhD and good research record in basic and laboratory endocrinology. Salary: \$A22,614 - \$A34,467 pa. Enquiries to Dr A Trounson, Centre for Early Human Development, Queen Victoria Medical Centre, 172 Lonsdale Street, Melbourne, Australia 3000. Applications including Ref No 32962, curriculum vitae and 3 referees to the Registrar, Monash University, Clayton, Vic 3168, Australia by 31/8/85.

An Equal Opportunity
 Employer
 (W2048)A

**Molecular Genetics
 — Leukaemia**

Applications are invited for a SHORT-TERM 3 YEAR appointment to the Council's non-clinical scientific staff. Candidates of immediate post-doctoral or equivalent status are preferred. The successful applicant will join a group studying the cytogenetics and molecular biology of radiation-induced myeloid leukaemia in the mouse and will investigate certain aspects of oncogene-activation in the disease. Experience in recombinant DNA techniques is essential. Salary according to MRC scales (equivalent to those of University non-clinical academic staff). Hostel accommodation is available. Applications, including full CV and the names of 2 referees, to the Personnel Officer (Ref. RC/HSE), MRC Radiobiology Unit, Chilton, Didcot, Oxon OX11 0RD. For further details phone Dr Roger Cox on Abingdon (0235) 834393. (7227)A

MRC Medical Research Council
 Radiobiology Unit

**ROYAL POSTGRADUATE
MEDICAL SCHOOL**
(University of London)
Department of Diagnostic
Radiology and Clinical
Pharmacology
TECHNICIAN

Technician is required to work on the development of fluorescent paramagnetic contrast agents for use in the measurement of renal and lung function. Both nuclear magnetic resonance and the measurement of biochemical and physiological parameters will be utilized to assess the function of these organs. One year's experience in general biochemical techniques and the possession of a Home Office licence would be an advantage. Candidates should have a degree or equivalent in a relevant subject.

The post is for one year in the first instance. Salary up to £5,795 plus £1,084 London Allowance.

Application forms and further particulars may be obtained from the Personnel Office, Royal Postgraduate Medical School, 150 Ducane Road, London W12 0HS quoting ref 10/T/N.

Closing date: 8 August 1985.
(7186)A

COLUMBIA UNIVERSITY

A tenure track position for a
**MOLECULAR BIOLOGIST,
IMMUNOLOGIST
or VIROLOGIST**

with guaranteed funding for five years and with joint appointments in the Department of Ophthalmology and the corresponding basic department of Columbia University, College of Physicians and Surgeons. Interested candidates should send their curriculum vitae and names of three references to Peter Gouras, MD, Chairman Search Committee, Columbia University, Department of Ophthalmology, 630 W 168 Street, NY 10032.

(NW61)A

**INSTITUTE OF
OBSTETRICS AND
GYNAECOLOGY**

(University of London)

HAMMERSMITH HOSPITAL

Ducane Road,
London W12 0HS

**POST DOCTORAL
RESEARCH ASSISTANT**

required to characterize hormonally sensitive proteins synthesised by normal and carcinomatous human endometrium in tissue culture.

Candidates should have relevant experience in biochemistry or cellular biology. Salary will be on the University of London scale according to age and experience. Applications in the form of a curriculum vitae including the names of two academic referees should be sent to Dr J. O. White at the above address. Informal enquiries can be made by telephoning (01 743 2030 ext 547). Closing date: 15 August 1985.
(7710)A

UNIVERSITY OF OXFORD
NUFFIELD DEPARTMENT OF
PATHOLOGY
RESEARCH TECHNICIAN

A research technician is required to join a group working on human leukaemia and lymphoma. The work will involve the use of immuno-enzymatic and DNA hybridisation procedures for the analysis of tissue and cell samples from cases of these disorders.

The post is supported by the Leukaemia Research Fund and it is for one year in the first instance. This grant represents the last year of a three year project and hopefully will be renewed on its expiry.

Salary on the Whitley Council medical Laboratory Scientific Officer Scale, the starting point to be decided on the basis of qualification and experience.

Applications, with the name of two referees to Dr D. Y. Mason, Haematology Department, Level 4, John Radcliffe Hospital, Headington, Oxford OX3 9DU, from whom further information may be obtained. Tel No: Oxford 817386.
(7211)A

BIOCHEMISTRY

POSTDOCTORAL POSITION

available immediately to study regulation of acetylcholine receptors in vertebrate skeletal muscle. Experiments involve preparation of subunit-specific antibodies and hybridization probes and measurement of levels of receptor polypeptides and their mRNAs, during denervation and reinnervation in vivo, and in response to specific stimulus regimes in vitro.

Applicants should have experience in recombinant DNA technology. Appointment for up to three years; starting salary \$19,000. Send c.v. and names of references to J. Schmidt, Dept. Biochemistry, SUNY Stony Brook, Stony Brook, NY 11794. SUNY Stony Brook is an affirmative action/equal opportunity educator and employer.
AK#171-85. (NW65)

**UNIVERSITY OF
EDINBURGH**

Department of Botany

**POST-DOCTORAL
RESEARCH ASSISTANT**

A post-doctoral research assistant, preferably with experience of plant tissue culture, is required to investigate the habituation of tissue cultures in relation to their competence to form flowers.

The post is funded by SERC for up to 3 years starting at £7,520 or £7,980 on the RA1A scale, and is tenable from 1st October 1985. Applications, with cv and the names of two referees, should be lodged by 20th August 1985 with Dr R F Lyndon, Department of Botany, University of Edinburgh, Mayfield Road, Edinburgh, EH9 3JH, from whom further particulars may be obtained. Please mark the envelope "SERC POST-DOC".

Please quote reference No 5267
(7191)A

CSIRO
AUSTRALIA

**DROUGHT RESEARCH/
OCEAN-ATMOSPHERIC
MODELLING**

\$A26,001 — \$A38,243

**DIVISION OF ATMOSPHERIC RESEARCH
ASPENDALE VIC**

GENERAL: The Division of Atmospheric Research has a staff of 130 including 70 professional scientists and undertakes research into physical and chemical aspects of atmospheric phenomena and related areas: The Large Scale Dynamics program is concerned with the identification of mechanisms which determine the general circulation in the atmosphere and their impact on climate and its variability. A major new initiative in this program is the expansion of our effort in the investigation of the causes of widespread Australian droughts using large scale models of the atmosphere and ocean.

DUTIES: To undertake research development of a numerical model of the ocean which can be coupled with a similar atmospheric model to provide a wide ranging climate research facility. The appointee will, in collaboration with other members of the team, design and conduct innovative experiments to investigate anomalous behaviour of the atmosphere — ocean system and its impact on Australian climate. Interaction with other CSIRO Divisions and organizations with mutual interests would also be required.

QUALIFICATIONS: A PhD degree or equivalent qualifications in Applied Mathematics or Physics with demonstrated research ability in oceanography or dynamical meteorology. An interest in the interannual variability of the atmosphere-ocean system together with experience in numerical modelling of the ocean or atmosphere is desirable.

APPLICATIONS: Stating relevant personal particulars, including details of qualifications and experience, the names of at least two professional referees and quoting reference No A1146, should be directed to:

The Chief
CSIRO Division of Atmospheric Research
Private Bag No 1
MORDIALLOC VIC 3195
by 4 weeks from date of publication

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER

(W2062)A

UNIVERSITY OF EDINBURGH
DEPARTMENT OF VETERINARY PATHOLOGY
WELLCOME TRUST LECTURESHIP IN MOLECULAR BIOLOGY

Applications are invited for a Wellcome Trust Lectureship in Molecular Biology in the Department of Veterinary Pathology, University of Edinburgh. The Department has a major interest and excellent facilities for basic research in immunology and the molecular aspects of pathology. The person appointed will join an active research group working on the immunology of persistent viral infection and will be expected to work on the molecular biology of pestiviruses (Border Disease virus of sheep, virus of Bovine Viral Diarrhoea — Mucosal Diseases of cattle) and investigate the molecular biology of the interactions between these viruses and the cells of immune systems in sheep. Molecular biology techniques have been introduced to the Department, and the successful applicant will be expected to further develop Molecular Biology in relation to the Department's research interests in immunology and its teaching programmes. Applicants must have research experience and a training in recombinant DNA technology and an interest in immunology.

The salary will be on the lecturers' scale (£7,190-£14,125). Starting salary depending on age, qualification and experience. The Lectureship is for three years in the first instance with renewal for a further two years on satisfactory review. Candidates should not be older than 35 years of age. Further particulars can be obtained from Professor I. McConnell, Department of Veterinary Pathology, University of Edinburgh, Summerhall, Edinburgh EH9 1QH (Tel: 031 667 1011 ext. 5322 or 5343). Candidates will be asked to submit a 500 word summary of the proposed research and should request the further particulars.

Applications (6 copies) giving the names of 3 referees should be sent to:

The Personnel Office, University of Edinburgh, 63 South Bridge, Edinburgh EH1 1LS.

Closing date for applications is 30th August, 1985, with appointment as soon as possible after that date. Applications from overseas should lodge their interest by this date with applications to follow.

Please quote reference 5266.

(7190)A

CSIRO

AUSTRALIA

RESEARCH FELLOW

\$A26,001 — \$A32,083

DIVISION OF PLANT INDUSTRY CANBERRA ACT

CSIRO conducts scientific and technological research in laboratories located throughout Australia and employs about 7,500 staff, of whom some 2,900 are professional scientists. The Organization's research activities are grouped into five Institutes: Animal and Food Sciences, Biological Resources, Energy and Earth Resources, Industrial Technology and Physical Sciences. The CSIRO Division of Plant Industry is a member of the Institute of Biological Resources.

FIELD: Plant cell culture/somaclonal variation.

GENERAL: The CSIRO Division of Plant Industry conducts research on problems fundamental to plant biology and agricultural production. Prominent in this research are programs concerned with gene transfer and expression, cell culture, photosynthetic processes and nitrogen fixation.

The primary objective of this project is to produce lucerne (alfalfa) genotypes containing condensed tannins in their leaves. Such plants would result in increased utilization of dietary protein and reduced bloat in grazing ruminants.

DUTIES: The appointee will undertake research to produce monohaploid lucerne via microsporogenesis or induced parthenogenesis. Variation will then be generated in populations derived from haploid protoplasts. The appointee will collaborate closely with a cell culture group of 7 scientists. Closely related projects include somatic hybridization studies with lucerne protoplasts. Collaborations will also be with animal nutritionists and lucerne breeders.

QUALIFICATIONS. A PhD degree or equivalent qualifications in a relevant field and preferably with research experience in one or more of the following areas: cell protoplast culture, another culture, legume genetics.

TENURE: A term of 3 years with Australian Government superannuation benefits available.

APPLICATIONS: Stating relevant personal particulars, including details of qualifications and experience, the names of at least two professional referees and quoting reference No. Y7723, should be directed to:

The Chief
CSIRO Division of Plant Industry
GPO Box 1600
CANBERRA ACT 2601
by 4 weeks from publication

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER

(W2060)A

POSITION ANNOUNCEMENT

The Department of Animal Science, Macdonald College of McGill University is seeking applicants for the position of

ASSISTANT PROFESSOR

in the area of

PHYSIOLOGICAL OR BIOCHEMICAL GENETICS

The appointment is a full-time, tenure track position. The successful candidate will be expected to develop a strong research program in an area of importance to animal science, such as regulation of growth, milk or egg production, or reproductive processes. The research should be complementary to ongoing programs within the Department in genetics, nutrition and reproductive physiology. A willingness to participate in interdisciplinary research projects and to teach in the areas of animal genetics and/or physiology at both undergraduate and graduate levels will also be expected. Qualifications include a Ph.D. in physiological or biochemical genetics with an orientation toward or experience in animal science. The base salary for an Assistant Professor is \$30,169. Closing date for applications is October 15, 1985 or until a suitable candidate is found. Interested applicants should send curriculum vitae and arrange for transcripts and at least three letters of reference to be sent directly to

Dr. Bruce R. Downey
Chairman, Department of Animal Science
Macdonald College of McGill University
21,111 Lakeshore Road

Ste. Anne de Bellevue

P.Q., Canada H9X 1C0

In accordance with Canadian Immigration requirements, this advertisement is directed to Canadian citizens and permanent residents of Canada.
(NW57)A

UNIVERSITY OF DUNDEE Department of Physiology RESEARCH ASSISTANT

Applications are invited from suitably qualified candidates for a Research Assistantship. The project, which is funded by the British Diabetic Association, is concerned with the control of transport into skeletal muscle and other tissues of amino acids and sugars in a variety of circumstances of health and disease. Tenure of the Research Assistantship will be for 2½ years. The successful candidate will be appointed on the Research Assistant IB scale starting at £6,600 up to £8,920. A possibility exists of registration for a higher degree. Candidates are invited to contact Professor M J Rennie (0382) 23181 extension 4572, who will supervise the project, for further particulars.

Applications (2 copies, or if airmailed from overseas, one copy) quoting reference EST/79/85J and containing full career details and the names of three referees should be lodged by 15th August 1985 with the Personnel Officer, The University, Dundee, DD1 4HN from whom further particulars are available
(7188)A

ST MARY'S HOSPITAL MEDICAL SCHOOL (University of London) Norfolk Place, London W2 1PG DEPARTMENT OF BIOCHEMISTRY POST-DOCTORAL RESEARCH ASSISTANT

Applications are invited for the above post to work on an MRC funded project on the microdissection and microcloning of the mouse X-chromosome. The project involves the construction of detailed molecular maps of individual regions of the mouse X-chromosome for the isolation and characterisation of interesting genetic loci, in particular mdx (X-linked muscular dystrophy). The applicant should have experience of recombinant DNA technology.

The post is available from 1 October 1985. Salary is on the 1A scale (starting point, £7,520 plus £1,233 pa London Allowance) for research staff.

Enquiries to Dr S. D. M. Brown, tel. (01) 723-1252 ext 333. Applications including full cv and names and addresses of 2 referees to Assistant Secretary (Personnel) at above address by 16 August 1985.

(7219)A

UNIVERSITY OF BIRMINGHAM

FACULTY OF MEDICINE AND DENTISTRY

DEPARTMENT OF RHEUMATOLOGY

RESEARCH FELLOW

Applications invited for a research fellow post, funded by MRC, to investigate the metabolism of iron by

MURDOCH UNIVERSITY Perth, Western Australia SCHOOL OF VETERINARY STUDIES LECTURESHIP IN MICROBIOLOGY

The appointee will be expected to teach general and pathogenic veterinary bacteriology, to develop a active research programme and to take part in the diagnostic microbiology service provided for clinicians. Training in bacteriology and a primary veterinary qualification would be preferred but the latter is not essential. Candidates with experience of training in contemporary molecular microbiology and/or immunology will be welcomed. Preference will be given to candidates with several years post-doctoral experience. (REF: 0453 — post available from January 1986).

General. Salary Range: \$A26,230 to \$A34,467 per annum (appointment will normally be made at the lower steps of the grade). This is a tenurable appointment and conditions include superannuation, long service leave, outside studies programme, payment of fares to Perth for appointee and dependent family removal and settling-in allowance and house purchase loan arrangement.

Procedure for applications: There is no prescribed application form but two complete sets of detailed applications, quoting the appropriate reference number, including full personal particulars, details of tertiary qualifications, career history with description of posts held, areas of special competence and interest research completed or currently being undertaken, personal views on teaching, membership of professional institutions or societies and positions of responsibility in these; list of relevant material published by the applicant, when available to take up appointment if offered and the names and addresses of three professional referees should reach the Personnel Officer, Murdoch University, Murdoch, Western Australia 6150, by 25 October 1985. Applicants should request the three professional referees to write immediately, quoting the appropriate reference number, to the Personnel Officer, Murdoch University, Murdoch Western Australia 6150. Applicants resident in the United Kingdom, Europe or Africa at the time of the application should also forward one further copy to: The Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.
(W2051)A

cells, and in particular the hepatocyte. Experience with tissue culture, immunological and/or biochemical techniques an advantage.

Salary on scale £7,520-£12,150 maximum starting £8,920 with superannuation for one year.

Further particulars Dr Stephen Young 021 472 1301 ext 3507

Applications (3 copies) to Assistant Registrar, Medical School, Birmingham B15 2TJ by 15 August 1985. Quote Reference RF/Rheum/SY (7222)A

YORK UNIVERSITY
DEPARTMENT OF EARTH AND
ATMOSPHERIC SCIENCE

**DYNAMIC
METEOROLOGIST**

The Department of Earth and Atmospheric Science at York University invites applicants for positions of University Research Fellow in the area of dynamic meteorology. The Fellowships are research positions funded by the Natural Sciences and Engineering Research Council (NSERC) at the level of Assistant Professor tenable for up to five years. It is expected that the successful candidates will be prime candidates for tenure stream appointments in the department as they become available in the next few years.

Further information can be obtained by contacting Prof. John C. McConnell, Chairman, Department of Earth & Atmospheric Science, York University, 4700 Keele Street, Downsview, Ontario M3J 1P3

These positions are tenable only by citizens or permanent residents of Canada at the time of application. In order to meet the NSERC deadline of 1st November, for fellowships to be awarded in 1986, York will require formal application by 30th September 1985. (NW49)A

**CHARING CROSS AND
WESTMINSTER MEDICAL
SCHOOL**

(University of London)

DEPARTMENT OF MEDICAL
ONCOLOGY

**BASIC GRADE
BIOCHEMIST**

Applications are invited for the post of Basic Grade Biochemist in the Department of Medical Oncology. The successful candidate will hold a degree in Biochemistry or related discipline and will work on an MRC-funded project using synthetic oligopeptides as immunogens for the production of anti-tumour antibodies.

Application forms available from The Secretary, Charing Cross and Westminster Medical School, The Reynolds Building, St Dunstan's Road, London W6 8RP, to be submitted within four weeks of the appearance of this advertisement. (7195)A

**AFRC INSTITUTE OF
ANIMAL PHYSIOLOGY**
Babraham Cambridge CB2 4AT
Two SENIOR SCIENTIFIC
OFFICER posts

Applications are invited for two New Initiative posts to work on regulation of animal cell growth. One post will be suitable for applicants trained in molecular biology and familiar with recombinant DNA technology including genomic library screening with oligonucleotide probes. The work will involve the characterisation of the genes for insulin-like growth factors. The second post will be for a cell biologist/biochemist to

**PUBLIC HEALTH
LABORATORY SERVICE**
CENTRAL PUBLIC HEALTH
LABORATORY
DIVISION OF ENTERIC
PATHOGENS
SENIOR GRADE
MICROBIOLOGIST

The Division is the National Reference Centre of Salmonella, Shigella and Escherichia coli, Vibrio and related organisms

The post holder will be involved in the investigation of the pathogenic mechanisms of these organisms.

Candidates should have a 1st or 2nd class honours degree in Microbiology and post-graduate experience is essential.

Further details from Dr S Scotland, ext. 3213.

Salary: £9,415-£12,173 plus £648 London Weighting

Closing date: 26th August 1985

For an application form and job description contact: Personnel Department, Central Public Health Laboratory, 61 Colindale Avenue, London NW9 5HT. Telephone: 01-200-4400. Please quote reference number DEP/2. (7197)A

**UNIVERSITY OF
NOTTINGHAM**

DEPARTMENTS OF
BIOCHEMISTRY AND SURGERY
POSTDOCTORAL OR
POSTGRADUATE
RESEARCH ASSISTANT

Applications are invited for the above post on a project funded by the Cancer Research Campaign to investigate biochemical and immunochemical aspects of glycoproteins produced by primary human breast cancers. The work will be carried out primarily in the Department of Biochemistry in close collaboration with Professor Blamey of the Department of Surgery. Initial salary will be up to fourth point on Range 1A for Research and Analogous Staff, depending on qualifications and experience.

Applications including a curriculum vitae and names of two referees should be submitted by August 1st to Professor F W Hemming, Department of Biochemistry, Queen's Medical Centre, Nottingham NG7 2UH (0602 700111 ext 3619). (7181)A

study the mechanism of action of these growth factors at the cellular level

The investigators will have support staff and will be expected to work in close collaboration with other scientists studying related areas. The appointments will normally be for three years with starting salary according to qualifications and experience within the pay scale £9,772 - £12,653 per annum. There is a non-contributory superannuation scheme. Application forms and further details are available from the Director's office quoting reference Phys/Bio/ST/NI. Closing date for applications 6th September 1985. (7189)A

CSIRO
AUSTRALIA

**RESEARCH SCIENTIST/
SENIOR RESEARCH
SCIENTIST**

\$A26,001 — \$A38,243
DIVISION OF FOSSIL FUELS
NORTH RYDE NSW

FIELD Ash Formation During Coal Combustion

GENERAL: The Division of Fossil Fuels forms part of the Institute of Energy and Earth Resources. The Division has a leading reputation in the science and technology of coal utilization, including the combustion of coal in power generating systems.

The Division is currently expanding its research into the coal preparation, combustion, and environmental control aspects of the use of pulverized coal in power plants. Particular emphasis is being given to the nature of formation, and properties, of the ash formed during combustion from the inorganic species present in the coal. The aim of the research is to develop strategies, based on sound understanding of fundamental principles and of practical combustion systems, to reduce problems due to ashes that foul and corrode, or erode, boiler tubes, and to help develop improved systems for the control of particulate emissions. The Division is at present increasing its experimental facilities to study pulverized coal combustion under controlled conditions ranging from 'single particle' studies to the use of simulated power station flames. A person is sought who will use his/her established research ability to make a significant contribution to the understanding of the fundamentals of the combustion process, and who will provide a significant input to the development of the Division's combustion research.

DUTIES: The appointee will carry out research on the physico-chemical processes undergone by the inorganic constituents of coal during combustion, in order to understand the manner by which ash is formed, and how the process of formation might be modified. The appointee will join an existing team at an early stage of work on coal ash, and will be expected to develop a leading role in this area. He or she will be encouraged to develop an advanced theoretical as well as empirical, understanding of the ash-formation process. Advanced combustion and diagnostic equipment, and computational facilities, will be available for use and development, and access will be given to chemical and physical analytical facilities, including optical and electron microscopes.

QUALIFICATIONS: A PhD degree or equivalent qualifications in inorganic chemistry, metallurgy, or chemical engineering plus demonstrated research ability. Research experience in the behaviour of inorganic species at high temperatures, or in coal combustion, is desirable.

TENURE: Indefinite with Australian Government superannuation benefits available.

APPLICATIONS: Stating relevant personal particulars, including details of qualifications and experience, the names of at least two professional referees and quoting reference No A3975, should be directed to.

The Chief
CSIRO Division of Fossil Fuels
PO Box 136
NORTH RYDE NSW 2113
by 4 weeks from date of Publication

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER

(W2064)A

IMPERIAL CANCER RESEARCH FUND
RESEARCH FELLOWSHIP

Laboratory of Molecular Pharmacology — Edinburgh

A position is available for a Molecular Biologist/Biochemist in the Laboratory of Molecular Pharmacology with Dr C. R. Wolf, for studies into the molecular basis of Chemical-induced cancer. The successful candidate will be involved in establishing a programme on the isolation of human genes coding for proteins involved in the activation and/or deactivation of chemical carcinogens. The regulation of the genes, within these multigene families, with important functions in this regard will be studied. Polymorphisms within these genes will be investigated in relation to cancer susceptibility. Three year appointment. Salary range £8,920 to £11,205, entry according to qualification and experience.

For further information please telephone Dr Wolf on 031-667 1011 ext 2417.

Applications with C.V. and names and addresses of three referees should be sent to Ms S. M. Hurley, Imperial Cancer Research Fund, Lincoln's Inn Fields, WC2A 3PX, quoting ref. 96/85. (7223)A

CSIRO
AUSTRALIA
RESEARCH FELLOW
PLANT PATHOLOGY
\$A26,001 — \$A32,083
DIVISION OF PLANT INDUSTRY
CANBERRA ACT

CSIRO conducts scientific and technological research in laboratories located throughout Australia and employs about 7,500 staff, of whom some 2,900 are professional scientists. The Organization's research activities are grouped into five Institutes: Animal and Food Sciences, Biological Resources, Energy and Earth Resources, Industrial Technology and Physical Sciences. The CSIRO Division of Plant Industry is a member of the Institute of Biological Resources.

GENERAL: The CSIRO Division of Plant Industry conducts research on problems fundamental to plant biology and agricultural production. One of the Division's programs involves research on viruses in plant rusts. The aims are to characterise these viruses using molecular techniques and to investigate the role these viruses may play in the development of rust disease. A new facet in this research is support from the Wheat Research Council to specifically investigate these viruses in wheat rusts and to develop probes for use in rust identification.

DUTIES: To undertake research on RNA viruses present in several different races and species of wheat rust.

QUALIFICATIONS: A PhD degree or equivalent qualifications with experience in the basic technologies of molecular biology. Experience with plant pathogens and/or double stranded RNA viruses would be an advantage.

TENURE: A term of 3 years with Australian Government superannuation benefits available.

APPLICATIONS: Stating relevant personal particulars, including details of qualifications and experience, the names of at least two professional referees and quoting reference No. E7725, should be directed to:

The Chief
CSIRO Division of Plant Industry
GPO Box 1600
CANBERRA ACT 2601
by 4 weeks from publication

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER

(W2059)A

CSIRO
AUSTRALIA
RESEARCH FELLOW
\$A26,001 — \$A32,083
DIVISION OF PLANT INDUSTRY
CANBERRA ACT

CSIRO conducts scientific and technological research in laboratories located throughout Australia and employs about 7,500 staff, of whom some 2,900 are professional scientists. The Organization's research activities are grouped into five Institutes: Animal and Food Sciences, Biological Resources, Energy and Earth Resources, Industrial Technology and Physical Sciences. The CSIRO Division of Plant Industry is a member of the Institute of Biological Resources.

FIELD: Molecular Biology of Symbiosis.

GENERAL: The CSIRO Division of Plant Industry conducts research on problems fundamental to plant biology and agricultural production. It maintains extensive programs in the biochemistry and physiology of biological nitrogen fixation by legumes and into the ecology of rhizobia and saprophytic diazotrophic bacteria. There is an active program on molecular aspects of plant and microbial genetics, including a newly-developed program supported by the Australian Wool Research Trust Fund involving the molecular biology of the legume symbiosis.

DUTIES: To undertake research on genetic exchange between rhizobia, particularly in the soil environment.

QUALIFICATIONS: A PhD degree or equivalent qualifications in a relevant field, preferably experience in molecular biology and/or microbial ecology, especially with rhizobia.

TENURE: A term of 3 years with Australian Government superannuation benefits available.

APPLICATIONS: Stating relevant personal particulars, including details of qualifications and experience, the names of at least two professional referees and quoting reference No Y7724, should be directed to:

The Chief
CSIRO Division of Plant Industry
GPO Box 1600
CANBERRA ACT 2601
by 4 weeks from publication

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER

(W2056)A

SCIENTIFIC ASSISTANT

\$16,037 yearly

Assistant scientists in conducting comparative research. Maintain laboratory equipment, analyze data and care for small live animals. Handle record keeping functions and other related duties.

Candidates must have BA/BS in Biology or Psychology, with sufficient research experience and/or training to carry out responsibilities described. A knowledge of histology would be helpful. Computer/word processing experience is desirable. To apply, send resumé to:

BOX NT 2703
810 Seventh Avenue
New York, NY 10019
An equal opportunity (M/F/H)
affirmative action employer

(NW70)A

**ST MARY'S HOSPITAL
MEDICAL SCHOOL**
(University of London)

Norfolk Place, London W2 1PG
BIOCHEMISTRY DEPARTMENT

RESEARCH ASSISTANT

The applicant would join a research team attempting to identify the genetic defect causing cystic fibrosis, and would be responsible for running the tissue-culture facility within the Biochemistry Department and maintaining cell-lines from normal and CF patients. He/she would also be actively involved in using cloned genes as inheritance markers in CF families.

The successful candidate will have a good honours degree; interest and experience in molecular biology and/or mammalian cell culture would be a great advantage.

Further particulars are available from Rosemary Akhurst (01) 723-1252 ext 117 or Brandon Wainwright (01) 723-1252 ext 24. Applications including full cv and names of 2 referees should be sent to the Assistant Secretary (Personnel) at the above address by 12 August 1985

(7183)A

**LUDWIG INSTITUTE FOR
CANCER RESEARCH**
CLINICAL ONCOLOGY
DEPARTMENT

St George's Hospital Medical
School, London, SW17
POST DOCTORAL
RESEARCH SCIENTIST

Applications are invited for the above post to work in the laboratory engaged in improving our understanding and treatment of human breast cancer.

The work will involve studies on mechanism of endocrine responsiveness. Experience in molecular biology and/or endocrinology would be an advantage.

The post will be for two years in the first instance. Salary scale £8,920 — £11,205 pa plus £1,233 pa London Weighting.

Applications should be in writing enclosing curriculum vitae and names and addresses of two referees to the Administrator, Ludwig Institute for Cancer Research (London Branch), Haddow Laboratories, Clifton Avenue, Sutton, Surrey SM2 5PX no later than 9th August, 1985.

(7185)A

**THE UNIVERSITY OF
MANCHESTER**

**POST-DOCTORAL
RESEARCH ON
MICROBIAL ADHESION**

A three-year M.R.C. funded post will be available in the Department of Bacteriology and Virology from October 1st for a microbiologist or a biochemist to work with Dr. P.S. Handley on a study of the adhesive and structural properties of chemostat grown *Streptococcus salivarius* cells. The project will investigate the growth conditions that control expression of cell surface structures responsible for adhesion. The appointee will set up a chemostat to produce cells for use in adhesion and electron microscope studies. Applicants should have previous experience in running chemostats and/or electron microscope techniques. Initial salary in the range £7,520—£8,920 p.a. Superannuation. Applications, with C.V. and names of two referees, to Dr P. S. Handley, Department of Bacteriology and Virology, Medical School, The University, Manchester M13 9PT, by August 16th

(7215)A

UNIVERSITY OF CANTERBURY

New Zealand

LECTURER IN ZOOLOGY

Applications are invited for the above position in the Department of Zoology from candidates qualified to teach and undertake research in cell biology. The appointee will be required to participate in the planning and teaching of a third year course in Animal Cellular Physiology. Applicants are asked to indicate their areas in which they would be able and willing to contribute to the teaching of zoology.

Applicants should arrange for three referees to forward confidential reports directly to the Registrar of the University. Candidates should also state the earliest date on which they would be able to take up the appointment.

The salary for Lecturers is on a scale from NZ\$23,622 to \$27,928 per annum.

Further particulars and Conditions of Appointment may be obtained from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 8PF.

Applications close with the Registrar, University of Canterbury, Private Bag, Christchurch, New Zealand, on 30 September 1985.
(W2054)A

UNIVERSITY OF ABERDEEN

DEPARTMENTS OF
BIOCHEMISTRY AND
AGRICULTURE

MOLECULAR BIOLOGIST/ BIOCHEMIST

Applications are invited for Molecular Biologists, Biochemists or persons qualified in other appropriate disciplines for a position as Research Assistant in Biochemistry to work with Dr N Hardman (Biochemistry Department) and Dr A. M. Paton (School of Agriculture) on a project supported by the BP Venture Research Unit, entitled "Artificially-induced symbioses in plants". The general aims of the project are to manipulate the biochemistry/genetics of a wide variety of plant systems by the introduction of micro-organisms using novel techniques. Previous experience in Molecular Biology/Nucleic Acid Biochemistry, or the practical application of recombinant DNA techniques would be an advantage. This is a three-year post, available from 1 October 1985, or date to be arranged.

Salary within the range 1A Scale £7,520-£12,150 or 1B Scale £6,600-£8,920 per annum for Research and Analogous Staff with appropriate placing (scales under review).

Further particulars and application forms from The Secretary, The University, Aberdeen, with whom applications (2 copies) should be lodged by 16 August 1985.
(7220)A

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE

(University of London)

DEPARTMENT OF PHYSIOLOGY

Three appointments on the
TECHNICAL STAFF

are available in the Department of Physiology to work in a Research Group investigating aspects of the nervous control of the circulation under the direction of Professor K. M. Sayer

Post 1 — A Senior Technician (permanent appointment U.G.C.-funded) to supervise the technical activities in two neurophysiological laboratories and one cardiovascular laboratory.

Post 2 — A Technician (5-year appointment B.H.F.-funded) to work in a neurophysiological laboratory and to be engaged in electronics and computing.

Post 3 — A Technician (2-year appointment M.R.C.-funded) to work in a cardiovascular laboratory.

All posts are tenable from 1 October 1985.

Further particulars and application forms available from School Office, R.F.H.S.M., Rowland Hill Street, London NW3 2PF (01-794 0500 ext. 4262). Closing date 9 August 1985 Please quote reference PHY/3T (7216)A

UNIVERSITY OF EDINBURGH

DEPARTMENT OF GENETICS

RESEARCH ASSISTANT

Applications are invited from Honours graduates in genetics, physiology or biochemistry to investigate the inheritance of growth, fatness and metabolism using diverse selected lines of mice.

The project is supported by the Agricultural and Food Research Council (AFRC) in collaboration with the AFRC's Animal Breeding Research Organisation and Poultry Research Centre and is under the supervision of Professor W. G. Hill.

The post will be for 3 years in the first instance. The salary will be on the Research Assistance (1B) scale, minimum £6,600 per annum and the successful applicant will be expected to study for a Ph.D. degree.

Exceptionally, an appointment on the post-doctoral Research Associate (1A) scale, minimum £7,520 per annum, may be made.

Further information may be obtained from and applications should be made to The Personnel Office, University of Edinburgh, 63 South Bridge, Edinburgh. All applications should include the names and addresses of 3 referees. Please quote reference 5268.
(7217)A

CSIRO AUSTRALIA

VIROLOGIST/ IMMUNOLOGIST

\$33,209 — \$45,083 pa

AUSTRALIAN NATIONAL ANIMAL HEALTH LABORATORY GEELONG VIC

The Australian National Animal Health Laboratory (ANAH) is a high security microbiological laboratory for the study of animal diseases exotic to Australia. It is located at Geelong, a city with population of 170,000 situated 80 kms south of Melbourne. Geelong offers an excellent lifestyle, with all the advantages of a major city in a semi-rural environment. Educational and recreational facilities are first class and ocean beaches are in easy range. When fully operational in 1985/86 it will employ a staff of 178, including 30 scientists.

ANAH plays an important role in diagnosis, research, training, and the development and testing of vaccines. There are currently four research groups within the Laboratory — Epidemiology, Molecular Virology, Pathogenesis and Immunity and Microbiological Security — which possess expertise in microbiology, immunology, veterinary pathology and molecular biology.

The Laboratory wishes to appoint a Virologist/Immunologist to the Epidemiology Group. The appointee will play a key role in the establishment of the Laboratory's capability for the diagnosis of exotic animal diseases; the Laboratory's research activities, particularly those aimed at improving diagnostic methods; and the training of field officers and laboratory staff in the diagnosis of exotic diseases

Applicants must possess a PhD degree or equivalent qualifications in microbiology or immunology and have a background of research experience and achievement in animal virology. A degree in Veterinary Science and eligibility for registration with the Veterinary Board of Victoria would be an advantage.

The appointee will spend one to three years in Thailand leading a small research team working on Foot-and-Mouth Disease soon after appointment.

Appointment is for an indefinite period and carries Australian Government superannuation benefits. Applications stating relevant personal and professional details, the names of at least three referees and quoting reference number A6760 should be directed to:

The Chief
CSIRO
Australian National Animal Health Laboratory
PO Bag 24
GELLONG VIC 3220
by 4 weeks from date of publication

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER

(W2066)A

MOLECULAR BIOLOGIST

The University of Texas System Cancer Center,
Science Park — Research Division

Applications are invited for a faculty position (Assistant or Associate Professor level) in a multidisciplinary research laboratory studying cellular and molecular mechanisms of carcinogenesis. Research programs at Science Park, located in Buescher State Park, 40 miles east of Austin, include somatic cell genetics, DNA repair and mutagenesis, carcinogen metabolism and adduct formation, tumor promotion, virology, mechanisms of lymphomagenesis, normal and tumor cell differentiation, biochemical genetics and growth factors. Candidates would be expected to have or establish an independent research program. Preference will be given to individuals who have extensive expertise and commitment in the area of molecular biology. The Science Park provides competitive salary and benefits, a stimulating research environment, postdoctoral and graduate students, and opportunities for interaction with faculty at nearby institutions including the University of Texas Health Science Center in Houston, University of Texas at Austin, and Texas A & M University.

Interested applicants should send a complete curriculum vitae, the names of three references and a brief statement of research interests to:

Dr Thomas J. Slaga, Director, UTSCC, Science Park—Research
Division, P.O. Box 389, Smithville, TX 78957
Phone (512) 237-2403

An Equal Opportunity/Affirmative Action Employer

(NW67)A

CSIRO AUSTRALIA RESEARCH FELLOW

(ENDOCRINOLOGIST)

\$A26,001 — \$A32,083

**DIVISION OF ANIMAL PRODUCTION
PROSPECT NSW**

FIELD: Immunophysiological Stimulation of Livestock Productivity

GENERAL: The Division of Animal Production with Headquarters at Prospect near Sydney, is increasing its research effort in the application of biotechnology and related research areas, eg Immunophysiology to Livestock. An important objective is to discover new ways by which the efficiency of animal production could be improved including, currently, manipulation of growth and quality of meat and fibre by immunophysiological methods.

DUTIES: As a member of a team concerned with the hormonal correlates of growth in livestock:

conduct research into the mechanisms by which peptide or protein hormones effect functions such as appetite regulation, digestion, protein and fat synthesis or energy utilization.

collaborate in interdisciplinary studies aimed at immunological regulation of hormonal factors believed to inhibit the expression of potential productivity.

QUALIFICATIONS: A PhD degree or equivalent qualifications in an appropriate field of science plus demonstrated research ability.

TENURE: Indefinite with Australian Government superannuation benefits available.

APPLICATIONS: Stating relevant personal particulars, including details of qualifications and experience, the names of at least two professional referees and quoting reference No. A0777, should be directed to:

The Chief
CSIRO Division of Animal Production
PO Box 239
BLACKTOWN NSW 2148

by 4 weeks from date of publication

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER

(W2063)A

CSIRO AUSTRALIA RESEARCH FELLOW

(Monoclonal Antibodies)

\$A26,001 — \$A38,243

**DIVISION OF PROTEIN CHEMISTRY
PARKVILLE VIC**

GENERAL: The Division of Protein Chemistry, with a staff of 118, conducts scientific research on the structure and function of proteins and their production via biotechnology and synthetic routes. The appointee will provide support to a number of research programs concerned with the characterisation of proteins and the development of methods for the production of proteins using recombinant DNA technology

DUTIES: The appointee will undertake research related to production of monoclonal antibodies and the characterisation of their binding. The antibodies will be produced in response to the requirements of the various research programs within the Division, and work will be generally carried out on a collaborative basis. The Division has an established hybridoma facility.

QUALIFICATIONS: A PhD degree or equivalent qualifications in a relevant field and demonstrated research ability. Experience in the production or use of monoclonal antibodies is essential. Applicants should preferably have prior experience in immunochemical techniques such as ELISA, Western transfer or immunohistochemistry

TENURE: A term of 3 years with Australian Government superannuation benefits available.

APPLICATIONS: Stating relevant personal particulars, including details of qualifications and experience, the names of at least two professional referees and quoting reference No. A1300, should be directed to:

The Chief
CSIRO Division of Protein Chemistry
343 Royal Parade
PARKVILLE VIC 3052

by 4 weeks from publication

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER

(W2065)A

CHAIRMAN AND PROFESSOR Department of Horticultural Science Ontario Agricultural College UNIVERSITY OF GUELPH

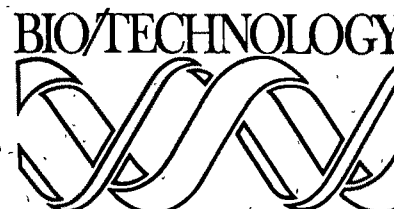
Nominations and applications are invited for the position of Chairman of the Department of Horticultural Science at the University of Guelph, Guelph, Ontario, Canada. The position becomes available July 1, 1986. The department offers an undergraduate major in Horticultural Science and a graduate program at the Masters and Ph.D. levels. It currently has 18 faculty and 35 Graduate students.

The department chairman will have administrative responsibilities for the departmental programs; provide leadership to the faculty and assist them in professional development; maintain liaison with other administrators within the university, with government agencies and allied industry groups to co-ordinate research, teaching, and extension programs and strengthen interdisciplinary activities; actively seek and develop new sources of funding for the departmental programs and take an active part in professorial organizations. The position provides the opportunity to continue personal involvement in teaching, research and extension. Candidates must have demonstrated leadership and administrative ability.

Applications will be accepted until October 31, 1985. Applicants should submit a curriculum vitae and the names of three people who may be contacted for letters of reference to: Dr F. L. McEwen, Dean, OAC, University of Guelph, Guelph, Ontario, Canada N1G 2W1. (519-824-4120, Ext. 2284). (NW63)

ATTENTION SCIENTISTS AND RESEARCHERS

Posters are now being accepted for
BIO/TECHNOLOGY's premier conference



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January 20-22, 1986, Clarion Hotel,
New Orleans

You are invited to submit abstracts of work with
commercial implications to:

Research Editor
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Ideas suitable for support from corporate or venture
funding are particularly welcome.

Conference Themes:

- Day 1: The Molecular Biology of Plants & Animals
- Day 2: Commercial Production Processes
- Day 3: Innovations in Pharmaceuticals & Immunology

UMIST

DEPARTMENT OF CHEMICAL
ENGINEERINGNEW BLOOD
LECTURESHIPSURFACE AND COLLOID
SCIENCE

Applications are invited for the above "New Blood" post. The person appointed will be expected to contribute to the science base of a wide range of research activities including mineral beneficiation, crystallisation, membrane technology and biochemical engineering. Further developments in these areas are largely dependent on a greater awareness of surface chemistry in order to achieve rapid and fundamental progress. A willingness to develop such interdisciplinary collaboration will need to be demonstrated by the successful candidates. Background in physical chemistry, surface chemistry or colloid science desirable.

The appointment will commence on 1 October 1985 or as soon as possible thereafter. Commencing salary will be within the scale £7,520-£8,595 p.a. (under review). Requests for application forms and further particulars, quoting reference E/41, should be sent as soon as possible to the Establishment Section, Registrar's Department, Room B9, UMIST, P.O. Box 88, Manchester M60 1QB.

Informal enquiries to Professor J. Marsden, Head of Chemical Engineering Department, UMIST, 061-276-3311, ext 2106. (7212)A

RESEARCH FELLOW: Position available for postdoctoral research fellow in protein biochemistry to study the relationship between peptide sequence and functional interaction of proteins in hemostatic mechanisms. May also involve some immunology and electron microscopy. PhD with strong background in protein chemistry preferred. Joint appointment will be held in an appropriate department at Harvard Medical School. Send resumé with details and two references to Dr Henry Sawyer, Laboratory of Structural Molecular Biology, Dana-Farber Cancer Institute, 44 Binney St., Boston, MA 02115. An affirmative action/Equal Opportunity Employer. (NW53)A

POST-DOCTORAL FELLOW: Required to work in area of human thyroid autoimmunity with emphasis on T-cell cloning. Opportunity for independent work and career development. Prefer individual with T-cell background, marine or human. Position available Summer 1985. Send resumé to T. F. Davies, Mount Sinai School of Medicine, 1 Gustave L. Levy Place, New York, NY 10029. An equal opportunity employer. (NW64)

UNIVERSITY OF KENT
AT CANTERBURY ■ ■ ■ ■

Biological Laboratory

Two Postdoctoral Research
Fellows in Yeast Molecular
Biology

Applications are invited for two Postdoctoral Research Fellowships (scale RA1A) to be held from 1 October 1985, for three years, in the Biological Laboratory.

Post 1: (supervised by Dr M F Tuite) The successful applicant will be involved in a project to isolate and characterise genes coding for the subunits of the protein synthesis initiation factor eIF-2 from *Saccharomyces cerevisiae*.

Post 2: (supervised by Dr M F Tuite and Dr K Gull)

The successful applicant will use site-directed mutagenesis to engineer cytoskeletal proteins of *Saccharomyces cerevisiae*, with a view to defining specific structure-function relationships.

Both appointments are SERC funded and salary will commence at £7,980 (at present being reviewed). We are looking for applicants who have already obtained, or are in the process of submitting a PhD, and who preferably have a working knowledge of molecular biological techniques.

Applications (two copies) giving details of age, qualifications and experience, together with the names and addresses of two referees should be sent to the Assistant Registrar, Faculty of Natural Sciences, Chemical Laboratory, The University, CANTERBURY, Kent CT2 7NH by 2 August 1985. Please quote ref A49/85/N. (7205)E

UNIVERSITY OF
MANITOBADepartment of Oral Biology
POSTDOCTORAL
FELLOWSHIP

Applications are invited for a Postdoctoral Fellowship to investigate the mechanism of action of bioactive peptides (SP, VIP etc) in controlling exocrine secretion in submandibular gland cells. Initially the project will concern the role of phosphoinositides. Starting date, spring 1986. Salary, around \$23,000.

Applications, including a cv and the names of two referees, should be sent as soon as possible to: Dr N Fleming, Department of Oral Biology, University of Manitoba, 780 Bannatyne Avenue, Winnipeg, Canada R3E 0W3. (NW62)E

IMPERIAL CANCER
RESEARCH FUND
RESEARCH FELLOWSHIP

A post-doctoral, three year appointment is available in our Cellular Pharmacology and Antitumour Chemistry Laboratory, to work with Dr A Creighton on projects involving tumour therapy with antibody targeted radionuclides.

Salary range £10,285 to £12,570, entry according to qualifications and experience. Further details are available from Ms S M Hurley, Imperial Cancer Research Fund, Lincoln's Inn Fields, London, WC2A 3PX, telephone 01 242 0200 ext 305 quoting reference: 93/85 (7147)E

FELLOWSHIPS

EMBO

EUROPEAN MOLECULAR BIOLOGY ORGANIZATION
SHORT TERM FELLOWSHIPS
in molecular biology

The European Molecular Biology Organisation awards, to scientists working in Europe and Israel in the field of molecular biology and allied disciplines, short term fellowships of one week up to three months duration. The fellowships are to support collaborative research between laboratories in different countries and provide a travel grant and subsistence allowance. Applications may be made at any time and are decided upon soon after the receipt of application.

Applications for exchanges between laboratories within any one country cannot be considered. Fellowships involving transatlantic travel are awarded only in extremely exceptional circumstances.

Application forms and further details may be obtained from Dr J. Tooze, Executive Secretary, European Molecular Biology Organization, 69 Heidelberg 1, Postfach 1022 40, F.R.G. (W1577)E

UNIVERSITY OF
NOTTINGHAM
MEDICAL SCHOOLDEPARTMENT OF
BIOCHEMISTRYPOST-DOCTORAL
FELLOW

Applications are invited for a research fellowship financed by the British Heart Foundation for a study of phosphoinositide metabolism and the molecular mechanisms mediating the responses of cardiac muscarinic receptors. The fellowship is tenable for two years initially from 1st October, 1985 at a starting salary of £7,980 or £8,450 depending on age and experience. Membership of USS included.

Applicants should send curriculum vitae and the names of two referees to Professor J N Hawthorne, Department of Biochemistry, Medical School, Queen's Medical Centre, Nottingham NG7 2UH, from whom further details may be obtained (telephone: 0602-700111 Ext 3488). Closing date 31st August, 1985.

(7065)A

UNIVERSITY OF WARWICK
POSTDOCTORAL
RESEARCH FELLOWSHIP
IN BIOLOGICAL SCIENCES

Applications are invited for an MRC-funded Postdoctoral Research Fellowship to join Dr Elizabeth Jones and Professor Hugh Woodland (MRC Animal Development Group) on a project involving the production of a panel of monoclonal antibodies to tissue-specific markers identifying the main embryonic tissues of the amphibian neurula. The antibodies produced will be used to study the cell biology of early cell determination. The candidate should have experience in tissue culture, preferably in hybridoma technology, or in developmental biology.

The position is for three years commencing October 1985 on the Research IA scale: £7,520 to £12,150 p.a. Applicants should send a C.V. (including a full list of publications) with the names and addresses of two referees to the Executive Officer, Department of Biological Sciences, University of Warwick, Coventry CV4 7AL, quoting reference EAJ/HRW/NAR. Further information can be obtained by ringing 0203-240111 ext. 6015.

Closing date. 8th August 1985.

(7196)E

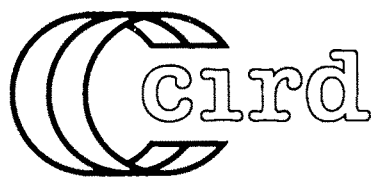
EMBO
European Molecular Biology Organization
LONG TERM FELLOWSHIPS IN
MOLECULAR BIOLOGY
Autumn 1985 - Awards
Next deadline: 15 August 1985

EMBO long term post-doctoral fellowships are awarded to promote the development of molecular biology and allied research in Europe and Israel. To be eligible a candidate must hold a doctorate degree and the exchange must involve a laboratory in Western Europe or Israel. EMBO fellowships are not, however, awarded for exchanges between laboratories within any one country. Long term fellowships are awarded initially for one year, but depending on a review of progress, they may be renewed for a second year. In cases of exceptional scientific merit renewal for a third year is possible. The fellowship comprises a return travel allowance for the fellow and any dependents and a stipend and dependents' allowance.

Since the selection procedure may include an interview, candidates are requested to respect the deadline for complete applications which is 15 August 1985. Successful candidates will be notified of their awards immediately after the meeting of the selection committee, which is on 21 October 1985.

Application forms and further details may be obtained from Dr J. Tooze, Executive Secretary, European Molecular Biology Organization, Postfach 1022.40, 69 Heidelberg 1, F. R. Germany. (W1893)E

SEMINARS & SYMPOSIA



The 6th CIRD SYMPOSIUM

"ADVANCES IN SKIN PHARMACOLOGY"

will be held at Sophia Antipolis, near Cannes
FRIDAY, OCTOBER 4 – SUNDAY, OCTOBER 6, 1985

TOXICITY AFTER TROPICAL APPLICATION

The cutaneous toxic effects of drugs applied to the skin will be evaluated. Aspects of percutaneous drug absorption as well as the biochemical consequences and side effects will be given prominence.

Friday Afternoon: In vitro Studies: Carcinogenesis

Marks, F. (Heidelberg), Gielen, J. (Liège), Bickers, D. (Cleveland), Chouroulinkov (Villejuif).

Saturday Morning: Animal Toxicology

Kemper, F. (Münster), Lombard, A. (Evreux), Oliver, G. (Macclesfield), Friedmann, J. C. (Paris).

Saturday Afternoon: Clinical Aspects including Penetration and Systemic Toxicity

Zesch, A. (Berlin), Schaefer, H. (CIRD), Maibach, H. (San Francisco), Lowe, N. (Los Angeles), Kligman, A. M. (Philadelphia).

Sunday Morning: In vitro Studies: Cytotoxicity in General

Reichert, U. (CIRD), Dunkel, V. C. (Washington, DC), Gilchrist, B. (Boston), Bernstein, I. A. (Ann Arbor).

For further information please write to Professor Hans Schaefer, Centre International de Recherches Dermatologiques, Sophia Antipolis, F-06565 Valbonne, France.

Telephone FRANCE (93) 95.70.70 — Telex F 461 030

THE AAD HAS ACCREDITED 14 HOURS CAT 1
 CME-CREDIT (W2053)M

ASSISTANTSHIPS

**UNIVERSITY OF HULL
 DEPARTMENT OF CHEMISTRY
 BP Extramural Research Award
 POSTDOCTORAL
 RESEARCH
 ASSISTANTSHIP IN
 SURFACE CHEMISTRY**

Applications are invited for the above post, initially for 1 year from an agreed starting date, to work with Dr R Aveyard on a project concerned with surfactant adsorption onto solids in oil + water systems exhibiting ultralow interfacial tensions, in connection with BP's interest in improved oil recovery. The group at Hull working under BP sponsorship will consist of 2 postdoctoral assistants and a PhD student. Applicants should have experience in colloid and/or surface chemistry.

The commencing salary will be in the range £7,520 — £9,390 per annum (under review) on the 1A scale for Research and Analogous Staff, depending on age and experience, plus USS benefits. Applications, together with the names of two referees and curriculum vitae, should be sent direct to Dr R Aveyard, Department of Chemistry, The University, Hull, HU6 7RX. (7184)P

**UNIVERSITY OF
 WARWICK**

**RESEARCH ASSISTANT IN
 BIOLOGICAL SCIENCES**

Applications are invited for an AFRC-funded Research Assistantship to work on a project concerning the molecular virological characterisation of rotaviruses. Applicants should hold a minimum upper-second class Honours degree, preferably with two years' postgraduate experience and be able to work independently. The successful applicant will not be eligible to register to work for a higher degree.

The position is for three years, commencing as soon as possible, on the research 1B scale: £6,600 to £8,920 p.a. Applicants should send a C.V. with the names and addresses of two referees, to the Executive Officer, Department of Biological Sciences, University of Warwick, Coventry CV4 7AL, quoting reference MAM/BS604. Closing date 15th August 1985. (7228)P

STUDENTSHIPS

**THE UNITED MEDICAL
 AND DENTAL SCHOOLS**

ST THOMAS'S CAMPUS

**STUDENTSHIP IN
 PHYSIOLOGY**

A Medical Research Council Studentship is available at the Sherrington School of Physiology. Applicants with a good degree in Physiology or related subjects should write with full curriculum vitae to Professor A. Taylor, Sherrington School of Physiology, St Thomas's Hospital Medical School, London SE1 7EH (tel. 01-928 9292 ext 2241) from whom further details are available. (7198)F

**PHARMACEUTICAL
 SCIENCES INSTITUTE**

ASTON UNIVERSITY

**CANCER RESEARCH
 CAMPAIGN**

**EXPERIMENTAL
 CHEMOTHERAPY GROUP**

**THREE RESEARCH
 STUDENTSHIPS**

are vacant immediately within this large, multi-disciplinary research group, funded by the Cancer Research Campaign.

(1) Study of the metabolism and mechanisms of hepatotoxicity of the anti-tumour agent N-methylformamide (Dr A. Gescher).

(2) Investigation of the effects of alkylating agents on the plasma membrane $\text{Na}^+\text{K}^+\text{Cl}^-$ cotransporter and its role in the control of proliferation (Dr J. A. Hickman).

(3) Biochemical investigation of factors responsible for weight loss in cancer (Dr M. J. Tisdale).

Candidates should have been awarded a first or upper second class honours degree in biochemistry, physiology, pharmacology or a related science. Enquiries should be made without delay to the CRC Experimental Chemotherapy Group, Aston University, Birmingham B4 7ET (021 359 3611, ext. 4175) (7202)F

CONFERENCES & COURSES

**COMBUSTION
 FUNDAMENTALS**

A one week course of post experience lectures will be held at IMPERIAL COLLEGE from 23rd to 27th September 1985.

Details from Professor F. J. Weinberg FRS, Department of Chemical Engineering & Chemical Technology, Imperial College, London SW7 2BY. Telephone: 01-589-5111 ext. 4360/4498. (7225)C

APPTS. WTD

DIPLOMPHYSIKER, DR.RER. NAT. from TU Berlin, Germany, Indian national, 5 years of research experience, 15 publications on radiation dosimetry and related fields; seeks suitable position in R&D. Box W2035 C/O Nature, 4 Little Essex Street London WC2R 8LF. (W2035)B

**THE ROWETT RESEARCH
 INSTITUTE**

Bucksburn, Aberdeen AB25

**RESEARCH
 STUDENTSHIPS**

(2 posts)

Applications are invited for Studentships leading to a PhD (Abercrombie University).

1. Research into the factors which influence rumen development and hence intake of dry feed young calves. This project, sponsored by British Sugars PLC, will investigate how the physiological/chemical mechanisms of the developing rumen respond to change the nature of the carbohydrate source supplied in weaner ration.

2. Research on in vitro systems of predicting nutrient digestibility feeds for pigs. This project, sponsored by external funds, involves the development of suitable chemical and biochemical techniques estimating the availability of nutrients in compound feeds for growing and adult animals.

Candidates for both positions should possess a first or upper second class honours degree in agricultural biochemistry, animal nutrition or related subjects.

Applications, including CV the names of two referees should be addressed to the Secretary, Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB, from whom further details can be obtained.

Closing date 8th August 1985. (7207)

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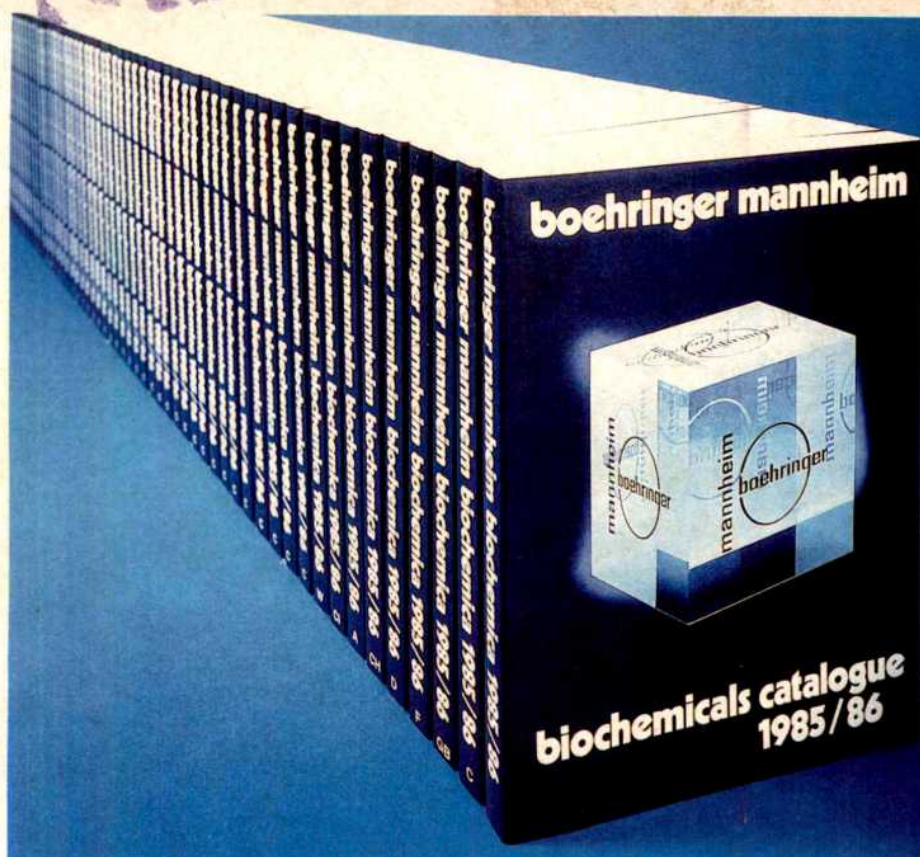
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Telex: 37155 ercodk dk

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6800 Mannheim 31
Tel. 0621/7591
Telex: 04-63193 bm d

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Telex: 980628 bmf test

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Boehringer Biochemia Robin,
Divisione Diagnostici
Via S. Uguzzone 5, Casella Postale 10217,
20126 Milano
Tel. 02/252 8356
Telex: 321 339 bmm i

JAPAN

Boehringer Mannheim Yamanouchi K.K.,
Kanda PO Box 168,
Tokyo
Tel. (03) 252-6521
Telex: 222-6244 alcaloj

THE NETHERLANDS

Boehringer Mannheim BV,
Postbus 1007,
1300 BA Almere
Tel. (03240) 94911
Telex: 70991 nl

NORWAY

Boehringer Mannheim GmbH
Biochemical Department
c/o Reidar Holmsen AS
Aslakveien 14 B,
0753 Oslo 7
Tel. (02) 50.31.65
Telex: 76971 xray n

SPAIN

Boehringer Mannheim S.A.,
Biochemicals,
Copérnico 61-63
08006 Barcelona
Tel. 93/7841722
Telex: 52589 bme-e

SWEDEN

Boehringer Mannheim Scandinavia AB,
Box 147,
16126 Bromma 1
Tel. 08/988150
Telex: 13054 bm scand

SWITZERLAND

Boehringer Mannheim (Schweiz) AG,
Industriestrasse,
6343 Rotkreuz
Tel. (042) 651165
Telex: 865256 bmch ch

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Bell Lane, Lewes,
East Sussex BN7 1LG
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Boehringer Mannheim Biochemicals,
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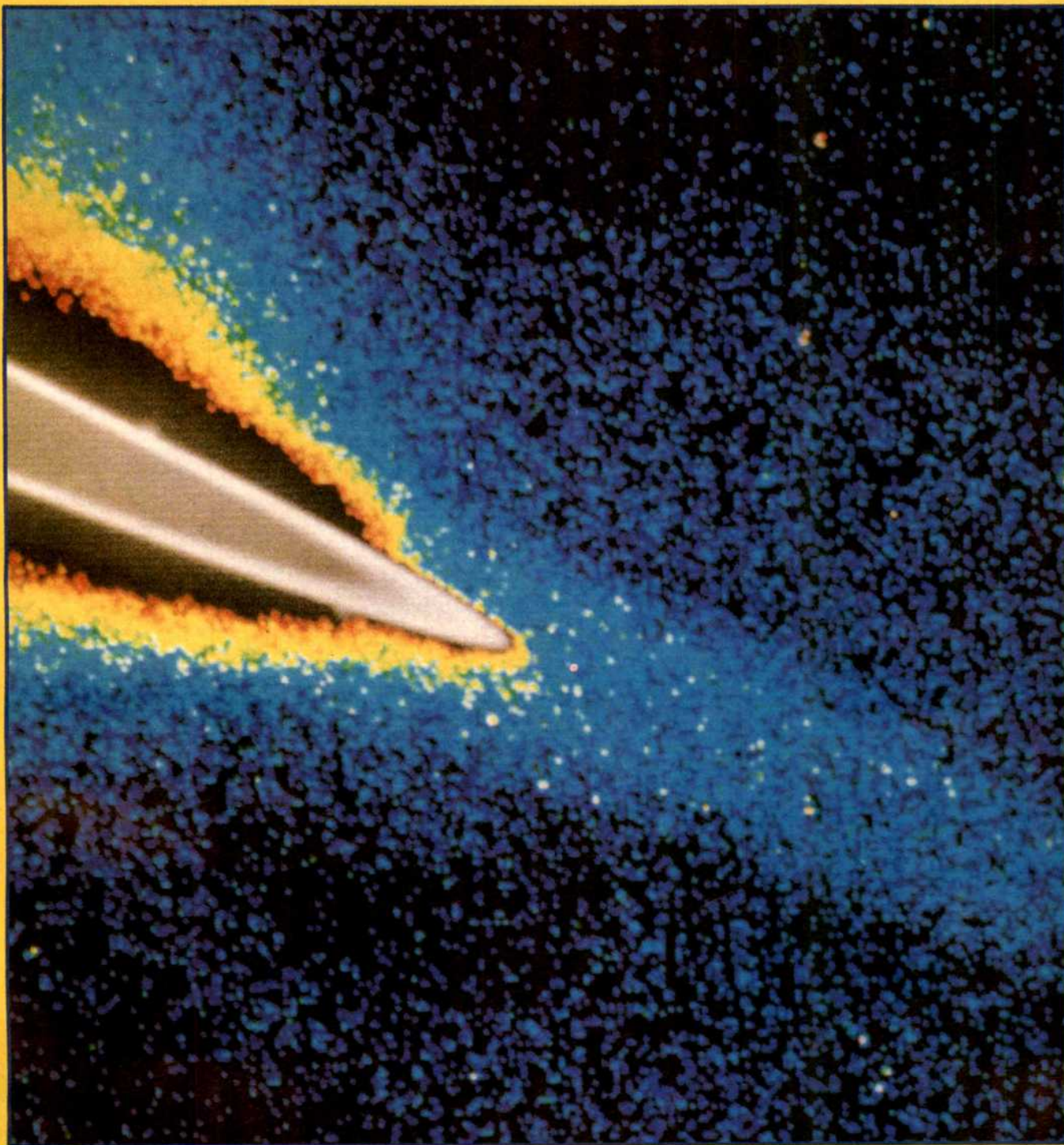
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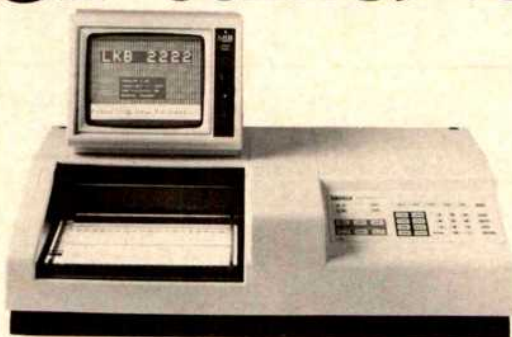
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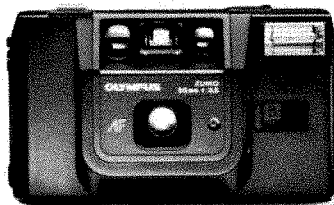
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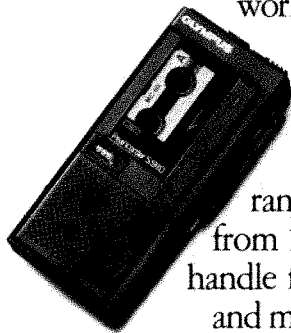
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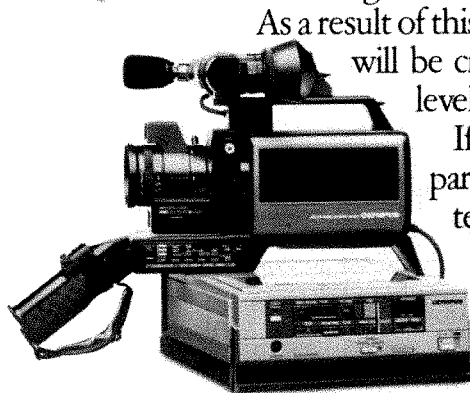
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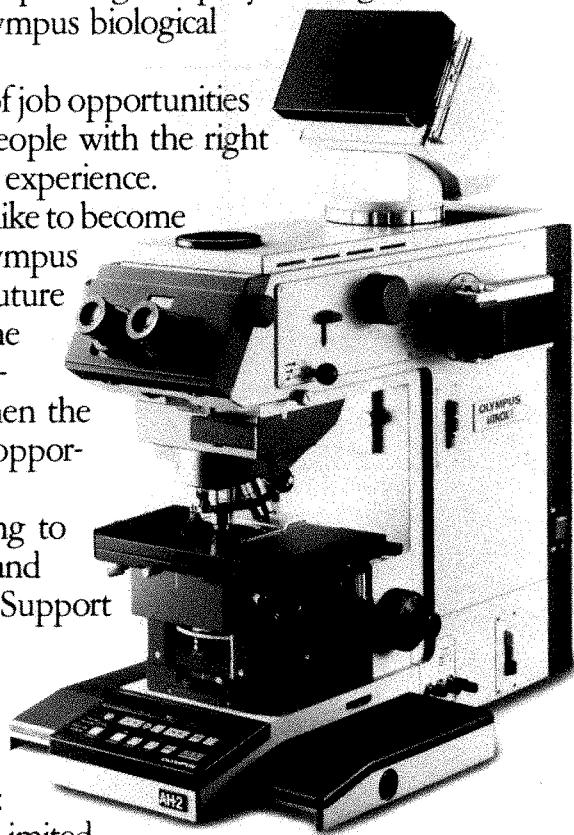
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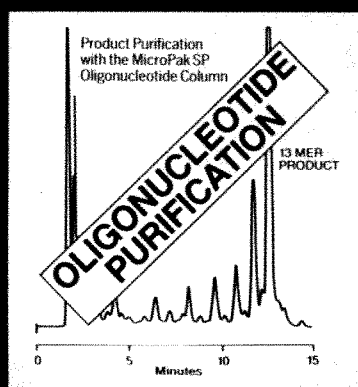
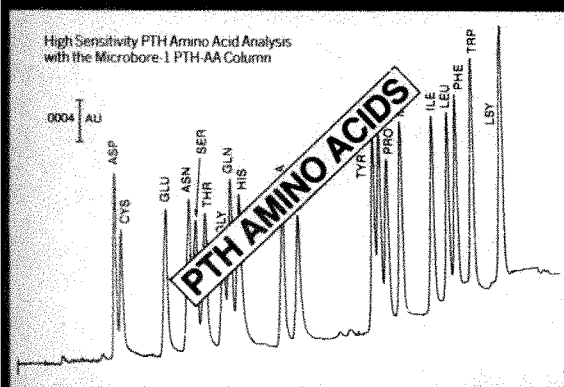
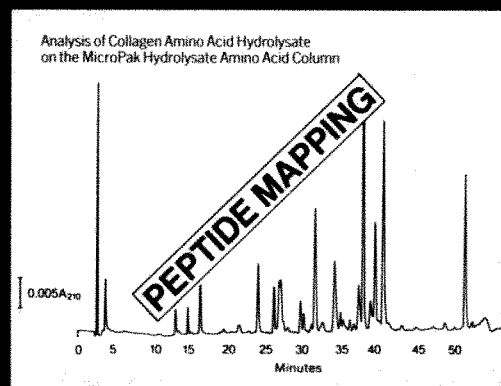
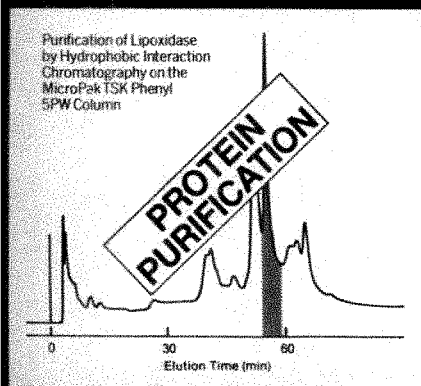
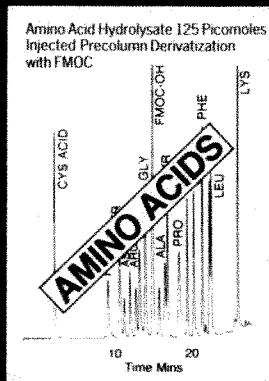
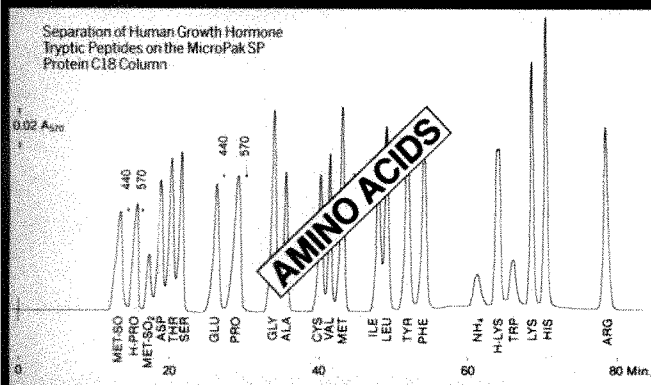


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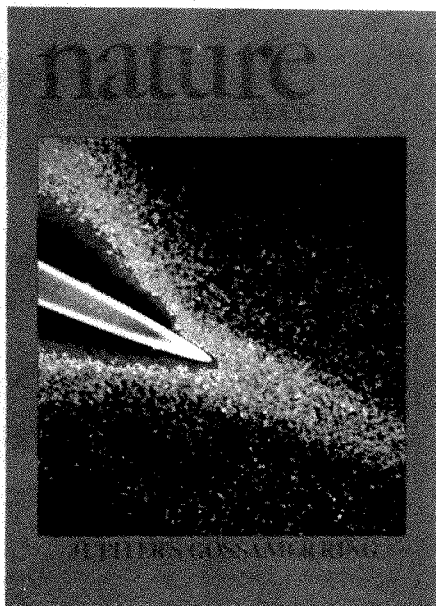
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An enhanced Voyager image of Jupiter's rings showing the bright ring (white), the halo (outlined in red-yellow) and the newly discovered 'gossamer' ring (a blue-green stripe running outward from the bright ring). See page 526.

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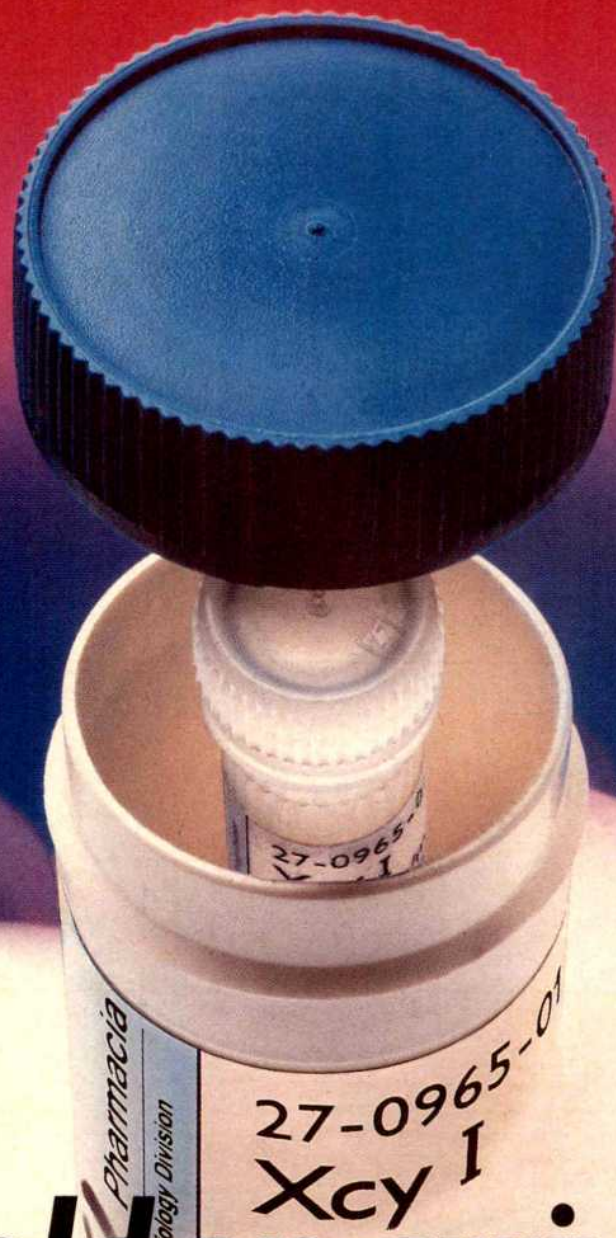
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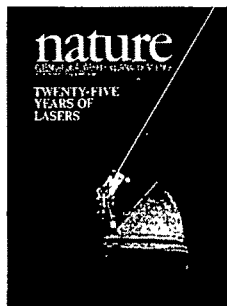
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Negotiating a test-ban treaty

The new-found excitement about a comprehensive test-ban treaty is premature. There are technical and military difficulties still to be surmounted.

THE fortieth anniversary of the Hiroshima explosion has seen an unexpected revival of interest in the notion that the testing of nuclear weapons might be banned altogether. Indeed, Mr Mikhail Gorbachev, the Soviet leader, some weeks ago announced a unilateral moratorium on testing even within the limits now allowed by treaty (only underground, and only with weapons yielding less than the equivalent of 150,000 tonnes of TNT). Mr Gorbachev also says that the Soviet Union will extend the moratorium indefinitely if the United States will only agree to follow suit. The new-found popularity of the comprehensive test-ban has been further emphasized by Sir Geoffrey Howe, the British Foreign Secretary, who was saying at the end of last month at Helsinki that the British government would be happy with a test-ban if only it could be properly policed. The only discordant note has come from the United States, where spokesmen in Washington have taken the line that a test-ban could not at present be monitored securely, for which reason the Soviet offer must be regarded as a kind of trap. As on many other recent attempts to make progress with arms control, everybody is half-right and, at the same time, half-wrong.

There are two reasons why the comprehensive test-ban has sprung to prominence just now. At the end of this month, the third review conference of the Non-Proliferation Treaty (NPT) will begin at Geneva, and the non-nuclear powers that have signed the treaty will be upbraiding the nuclear powers for having done so little, these past fifteen years (since the treaty came into force), to negotiate measures to limit strategic arms. The Soviet Union can hope to fend off at least some of the criticism by pointing to Mr Gorbachev's offer. Soon afterwards, in November, Mr Gorbachev and President Ronald Reagan will be meeting again at Geneva; the former is certain to ask the latter why the United States will not agree to a comprehensive test-ban treaty. Both sides had better acknowledge in advance that the issue between them will not be simply settled. In many ways, the technical problems are not the most obdurate.

Workable

Close on seven years ago, the three signatories of the threshold test-ban treaty, Britain, the Soviet Union and the United States, had all but settled the terms of a workable treaty. Then, as now, it was plain that a comprehensive treaty cannot be verified by the recording of seismic signals picked up by seismographs located entirely outside the territory of the signatories to such a treaty, whence the sensible technical compromise that signatories should each provide houseroom for ten remotely operated seismic stations on their own territory. The haggle that had arisen during the negotiations of this draft treaty about the suspicion that such stations might be used for more general espionage were eventually resolved, apparently amicably, by the US suggestion that public-key cryptography could be used to provide security; the host country would be able to decode messages, but would be unable (lacking the encoding key) to corrupt them with false information. It may be that, on reflection, the United States doubts the validity of these solutions, but there is no reason to fear that the problems tackled seven years ago are not soluble in one way or another, perhaps by refining the network of observing stations, perhaps by more elaborate ways of making the transmission of information secure. (The absurd Soviet demand that there should be ten seismic stations

in the United Kingdom and its possessions is a different kind of stumbling-block, but one that is, as it was, negotiable.) What is lacking now is the enthusiasm for a comprehensive test-ban.

What has gone wrong? The Soviet Union appears to have forgotten that the eagerness of the United States to conclude further arms control agreements had waned long before the present US administration came to power at the beginning of 1981, and that the decisive Soviet influence over Afghanistan was as much responsible as President Carter's recognition, during his last year in office, that it would not be possible to force the SALT II agreement through the US Congress. Since then, East-West relations have further deteriorated, both in rhetoric and by the evidence of events. During the same period, the United States has made the painful discovery that the single new strategic missile allowed by the SALT II treaty, the MX missile, is too clumsy an object for security, and has also argued loudly (if mistakenly) that it should be possible to avoid the threat of nuclear attack, and also of the need of specific arms control agreements, by means of a defence against ballistic missiles. The Soviet Union may say "bad luck!" on the first score and be sceptical to the point of hostile disbelief on the second, but it must also know that two sovereign states can be made to conclude a treaty only if each should voluntarily have concluded that its own interests will be advanced by whatever surrender of sovereignty is entailed.

Unpropitious

There are other reasons why the present time is not propitious for a comprehensive test-ban treaty. With the passage of almost a decade since negotiations on the existing draft began, nuclear powers not party to the negotiations, France and China specifically, have become a decade more sophisticated. China's accumulation of nuclear explosives has probably been proportional to the time elapsed, but China has no plutonium warheads as things are; the chances that such a power would sign a comprehensive test-ban treaty are small, which should give the Soviet Union pause. France is less of an immediate problem, but cannot entirely be ignored. None of this implies that these two nuclear powers should be left entirely to their own ambitions, but the process of discovering where they stand cannot be accomplished by the issuing of public challenges by one superpower to the other.

Another reason why the test-ban treaty is more than a matter on which all people of good intentions should instinctively agree turns on the uncertainty that would inevitably be engendered among the military people, and thus among politicians in both East and West, if the testing of nuclear weapons were suddenly outlawed. Will those warheads function as we designed them? Quite apart from the nuclear explosives, will the other components still do their job? A test-ban treaty would be quickly followed by the development of techniques for the near-testing of nuclear weapons, but uncertainty about the function of existing stockpiles would persist. That is why, in present circumstances, a comprehensive test-ban treaty is attainable only as part of a larger package, involving a substantial reduction of the numbers of warheads deployed by the two superpowers. The occasion to look for such an agreement is not next November's summit but the bilateral negotiations that will have resumed a good two months earlier. □

Vaccine-damage damage

A scheme to compensate for vaccine-damage in the United States must be delayed no further.

WHEN and how should individuals be compensated for injuries they suffer in the interests of the public good? This is the question underlying the thoughtful report last week from the Institute of Medicine in the United States, grappling yet again with the question of how the occasional injuries caused by vaccination should be dealt with (see p.476). The rationale of mass vaccination programmes is that the treatment of individuals will not merely protect them against an infection but also help break the chain of infection in the population at large. There are by now many spectacular illustrations of the success of vaccination, the chief agent in the eradication of smallpox and the reduction of the incidence of the damaging paediatric infections, at least in the industrialized countries of the world.

But vaccines are not free from risk. Even when properly manufactured, they may damage those who look to them for protection. The Sanford committee catalogues the social and legal anomalies occasioned by this ironical contradiction, advocating a statutory compensation scheme for those affected. In doing so it follows a long line of private organizations that have urged the need of similar arrangements (already in place, on a modest scale, in California). For at least the past three years, the US Congress has been grappling in a desultory fashion with legislation designed to give effect to such a proposal; in the Senate, the chief sponsor has been Senator Paula Hawkins, in the House of Representatives, Mr Henry Waxman and Mr Edward R. Madigan have taken the lead. The sponsorship is far from negligible; why has nothing happened?

The difficulty seems to be that all the participants in the administration of vaccination programmes and their consequences are ambivalent. Manufacturers of vaccines seek above all to escape from the present uncertainty, in which they (and their insurers) cannot guess in advance the monetary scale on which they might be liable if a vaccine should provoke a rash of lawsuits, but would not wish to go so far as to become subcontractors for some agency of the US government, which might then become the sole source of vaccines and the one responsible for compensation. The federal government, although anxious that vaccination programmes should not be hamstrung as at present, is similarly concerned about the cost (witness its experience with the epidemic of swine influenza that never came, in 1976) and the implication that this small corner of medicine might be "socialized". Physicians who administer vaccines, solicitous for their patients, have an interest in seeing that manufacturers and not themselves are usually held responsible for accidents. Can practising, as distinct from academic, lawyers have an interest that the present jungle of the courts should be perpetuated?

The resolution of these conflicting interests should be much easier than the Institute of Medicine allows. What seems to frighten everybody is the high cost of settling damage suits in the US courts (or in the corridors around them) and the difficulty, apparently often equated with constitutional impropriety, of interfering with the law of tort which allows an injured person to sue whoever may be responsible. But it is now made clear that in the settlement of vaccine damage suits, the amount of compensation paid to those who are tragically injured, or to their relatives, will often include a large element intended to compensate for "pain and suffering" occasioned by the accident. It is right and proper that manufacturers distributing defective vaccines should be sued for whatever the courts will award, but otherwise, given that the risks of vaccination are in any case not negligible, but that those consenting to vaccination do so in the expectation of personal immunity from some infection, it would be more equitable than the present system that the right to sue should be replaced by the right to administrative compensation, provided that the system offered as an alternative is both speedy and sufficient to cover money losses, medical expenses and support for dependants, for example. The Sanford committee

might usefully have grasped this nettle. It should also have pointed out that its useful compilation of vaccine-damage schemes in place elsewhere than the United States is not as valuable a guide as it may seem; the British scheme, for example, which limits administrative compensation to £10,000 but which does not extinguish the right to sue the manufacturer of a vaccine, is more generous than it may seem because of the free care provided by the National Health Service for those who may be damaged.

So why shrink from meddling with the law of tort in the United States? Vested interests (those of the lawyers and of the litigious) apart, one of the chief inhibitions seems to be the knowledge that the whole apparatus of case-law on product liability built up by the US courts would be undermined by the acknowledgement that a person's right to compensation would be mitigated by the expectation that the purchase of a disputed product would ordinarily be beneficial. Provided that the purchaser knows that there are also risks, the doctrine is equitable; but plainly it also applies to products other than vaccines, pharmaceuticals for example. And when there is public as well as private benefit involved, it is not merely necessary but desirable that the state, or the federal government in the United States should be directly involved in holding the ring between public and private needs. The Sanford committee rightly draws attention to the analogy between vaccine programmes and the arrangement for the insurance of nuclear power stations by the Price-Anderson Act, where the same principles are (or were once thought to be) at stake. The need now is that the US Congress should buckle down to the legislation it must know it cannot put off much longer. □

Growing charitable

Charitable foundations in the United States and Europe are learning how to flex their muscles.

EARLIER this year, the Howard Hughes Foundation leapfrogged its way to the top of the donors' league in the United States by divesting itself of TWA, the airline that was its founder's chief legacy. In due course, the result will no doubt make the foundation a force to be reckoned with in biomedical research. Although the charity's annual income will be less by more than an order of magnitude than that of the US National Institutes of Health (NIH), it will have the advantage of being uncommitted to the standing army of in-house researchers that NIH maintain and free from obligations wished on it by the US Congress. And now, independently and more cautiously, the Wellcome Trust in Britain proposes to take a leaf out of the Howard Hughes book. The two charities resemble each other in that their chief asset has from the beginning been the sole ownership of a commercial company, in Wellcome's case the drug house called confusingly the Wellcome Foundation. Next year, the trust plans to sell a fifth of its shareholding in the company, calculating that it will as a consequence be able to increase its spending on biomedical research by something like a factor of two. Already, the trust's grant-making, planned at £30 million during the year ahead exceeds what the British Medical Research Council can afford by way of research grants.

But why stop at the disposal of a mere fifth of the shareholding? Why not sell the lot, as the Howard Hughes Foundation sold the whole of the airline that was its birthright? The reason why it is financially advantageous for foundations to dispose of assets that consist of single companies is that, even when the companies are successful, they will sacrifice income (and their charitable objectives) for the chance of capital growth. But on the principle that there is no such thing as a free lunch, capital growth is not cast-iron. Even successful companies risk falling on hard times, as the Nuffield Foundation, once Britain's biggest, learned to its cost when, having sold a third of its shares in the British Motor Corporation, it found the rest bought by the British government at a knockdown price. Wellcome should sell more soon. □

Supercomputers

US seeks restrictions on foreign access

Washington

THE United States is pressing allied governments to restrict access to supercomputers by Soviet bloc nationals. Defense Department officials fear that access to the latest generation of machines could allow the Soviet Union to make significant advances in supercomputer technology and programming, or even to run programmes with military applications.

The new generation of supercomputers, exemplified by the Cray 2 or the Cyber 205 models, works at speeds in the gigaflop range, of the order of 10^9 floating point operations per second. The Soviet Union has no machines capable of this speed, which are made exclusively in the United States and Japan. Some employ innovative "vectorizing" software that allows limited parallel processing; and they can be used to test experimental parallel processing software that will lead to yet further advances in computing speeds.

Restrictions on access are likely to be no more popular with foreign than with US academics however. An acrimonious public dispute erupted last month because the National Science Foundation (NSF) asked several universities hosting NSF-financed "supercomputer centers" to ensure that citizens of COCOM-proscribed countries are denied access.

Only the centre at the University of California at San Diego agreed and, even there, officials say the matter has not been laid to rest. (The contract was actually signed by an industrial representative, and officials hint that things might have been different if the university had known more in time.) The centre at Princeton University made a curious compromise by volunteering to impose restrictions but only if required to do so by law, and two of the centres, at Cornell and Illinois, refused. NSF backed down, and officials now acknowledge that to impose restrictions without a national policy on access was "a mistake".

An attempt is being made to resolve the issue by an interagency group under the National Security Council; officials say that the academics will be heard, but at least one, Don Goldstein of the Department of Defense, contends that controls of some type will be necessary and that they should be in place within weeks. If no agreement is reached with universities, control might be achieved by denying entry visas to nationals of some countries planning to work on US supercomputers.

The Soviet Union is seen as the main threat, but there is also some concern about China, according to Goldstein. And he points out that it would be pointless to

impose controls on access in the United States while allowing unrestricted access to similar machines overseas.

Although supercomputers, running up to one hundred times faster than previous top-of-the-line models, can make otherwise daunting problems feasible, officials

Space cooperation

Summit speeds US/Soviet goals

Washington

No firm evidence has yet emerged of a decision by the US administration to pursue formal cooperation in space with the Soviets, despite a flurry of recent interest and the efforts of several US Senators who want President Reagan to make new proposals at his November summit with Soviet leader Mikhail Gorbachev. At recent hearings in Congress, a State Department official spoke of the desirability of renewed cooperation "down the road" but avoided making a commitment to renewing the agreement on cooperation in space that was allowed to lapse following the imposition of martial law in Poland.

At present there are a number of low-level contacts and exchanges between US and Soviet space scientists. Some US instruments are being flown on the Soviet Vega missions to comet Halley, for example, as they were on some of the Soviet Cosmos series of biosatellites. But there are no formal collaborations between the two countries directly, despite last year's Joint Resolution of Congress, signed by the President, committing the administration to work towards increased cooperation. A proposal last year by President Reagan for a joint simulated space rescue has remained unanswered.

Many believe there will be no significant upswing in cooperation with the Soviets until there is a new agreement; Soviet scientists do not have the same degree of latitude to "freelance" on joint projects as their counterparts in the United States. The earlier space cooperation agreement arose at the Nixon/Brezhnev summit in Moscow in 1972, which may thus be a precedent for next November.

The case for a joint Mars project is being pushed most notably by the Planetary Society, which together with the American Institute of Aeronautics and Astronautics held a meeting here last month to celebrate the tenth anniversary of the Apollo-Soyuz test project. The meeting was attended by Soviet cosmonauts who participated in the project, as well as by officials of the US National Aeronautics and Space Administration

admit it is unlikely that long-running programmes for submarine tracking or weapons design could be run clandestinely at a supercomputer centre.

Nevertheless, they say, the damage would be high should anyone succeed. And to be consistent, there will have to be restrictions on access to private supercomputers similar to those being talked about for academic institutions. Most of the world's 140-odd supercomputers are in the United States, and at present there seem to be no regulations limiting access to those owned by, for example, computer services companies. **Tim Beardsley**

(NASA). Significantly, NASA's administrator, James Beggs, gave his blessing to the idea of a manned Mars mission as a long-term goal.

The technical obstacles facing a manned Mars mission are indeed formidable, and NASA officials are quick to point out that there are no immediate plans for such an ambitious undertaking. More modest cooperation with the Soviets is thought more likely. NASA officials note approvingly that visiting Soviet scientists have recently been much more open about future Soviet space missions, and Dr Frank McDonald, NASA's chief scientist, expects attempts to coordinate with the Soviets over unmanned Mars missions planned for the end of the 1980s.

The Soviets have volunteered many details of their planned 1988 mission to the Mars moon Phobos, for example, and McDonald thinks there would be clear advantages to both sides in designing complementary instrumentation and if data were shared with a US Mars observer mission planned for 1990. A group of senators led by Spark Matsunaga has urged President Reagan, in legislation now pending, to raise the question of cooperation on Mars exploration with Gorbachev in November.

Congressional supporters of increased cooperation have recently been given some new ammunition in the form of a technical memorandum from the Office of Technology Assessment*. The memorandum notes several areas where cooperation with the Soviets has yielded technical benefits in the past, particularly the effects of long-duration space flight on astronauts and data about the surface of Venus. Despite American concerns about technology transfer, the memorandum concludes that there is potential for future cooperation on "global habitability", exobiology, Antarctic meteorites and some areas of astrophysics, as well as planetary exploration. **Tim Beardsley**

*US-Soviet Cooperation in Space a technical memorandum Office of Technology Assessment, July 1985.

AIDS

Two British blood tests launched

THE first stage of the British government's evaluation of commercially available kits to screen blood for the AIDS (acquired immune deficiency syndrome) virus, HTLV-III (human T-cell lymphotropic virus), was completed last week. The Virus Reference Laboratory of the Public Health Service has tested five manufacturers' kits (Abbott Laboratories Ltd, Electronucleonics Ltd, Organon Teknika Ltd, Ortho Diagnostic Systems and Wellcome Diagnostics). Three performed appreciably better than the others in distinguishing clearly between negative, positive, false-positive and heat-treated sera. Two of these, from Wellcome (Britain) and Organon (the Netherlands), were found to be especially easy to use and are now to be tested by the Blood Transfusion Service on about 6,000 samples. When this evaluation is complete, routine screening of blood donations will be introduced, probably by mid-September.

Screening will protect those most vulnerable to the AIDS virus, that is, people dependent on blood transfusions. For the present, "at risk" individuals have been asked not to donate blood. But AIDS is unlikely to stay contained within some sections of the population. Already there are reports of the presence of antibodies to the virus in women in some African cities, confirming that the disease can be transmitted by heterosexual contact.

Although the kits will greatly increase the efficiency of detecting the AIDS virus in sera, and hence containing its spread, a cure for the disease is no closer, although recent results indicate that the drug Zidovudine can help patients with AIDS-related complex or lymphadenopathy syndrome.

The AIDS virus replicates within the body's immune system and, although it is highly infectious, only 3 per cent of people infected with HTLV-III develop AIDS; however, another 20 per cent develop illnesses of varying severity ranging up to persistent generalized lymphadenopathy.

AIDS is almost always fatal, but the virus has a long incubation time. In July 1985, about 10,000 people in the United Kingdom and a million in the United States were estimated to be infected with HTLV-III. Until treatment is available, the first step in management of the condition is to prevent the spread of infection by screening blood for HTLV-III antibodies. The kits evaluated last week accurately identify the presence of HTLV-III antibodies in the blood. The next development will be to develop a test for the antigen, which will allow earlier identification of the virus, before an individual has made antibodies. It is hoped that antigen tests will be easier to perform and more sensitive than antibody binding.

Of the tests chosen for further evaluation in Britain, Wellcome's is a competi-

tive enzyme-linked immunosorbent assay (ELISA) and Organon's, in common with the unsuccessful tests, is an indirect ELISA. The Wellcome kit consists of a plastic well first coated with "control" HTLV-III antibody and then with impure HTLV-III antigen. (This procedure purifies the antigen.) The user adds to the well a test sample of serum together with a conjugate of control antibody linked to the enzyme horseradish peroxidase. HTLV-III antibodies in the sample compete with the enzyme-linked antibody in binding to the antigen. Unbound components of the mixture are washed out, and the substrate of the enzyme added to the wells. Any enzyme still bound to the wells is seen as a blue colour, and when the reaction is stopped with sulphuric acid, a yellow colour develops. If antibodies were present in the sample, some will have bound to the antigen; because they are not enzyme-linked, less colour develops.

The indirect ELISA also starts with antibody-antigen-coated wells. The sample is then added, washed, then enzyme-linked human anti-immunoglobulin G (IgG) is added to the mixture. Any antibody in the sample will bind to the anti-IgG and the development of colour indicates HTLV-III antibodies are present.

In both tests, samples scoring positive are subjected to a confirmatory test, such as Western blotting, in which the constituent proteins of the virus are separated on a two-dimensional gel and identified by their relative molecular mass.

The Wellcome test may have an advantage over the indirect ELISA because there are fewer steps (five against Organon's eight and taking two hours rather than just under three), there is not as much sample dilution and it is potentially more specific as it uses the principle of competition rather than binding to a more general antibody. However, the National Institute of Biological Standards and Control has found no difference in specificity between the two successful tests on a limited number of samples. **Maxine Clarke**

AIDS

US blood-bank tests established

Washington

SECOND-generation tests for the AIDS (acquired immune deficiency syndrome) virus that may reduce the persistent problem of false positives have produced encouraging early results. Abbott Laboratories, Centocor and Travenol-Genentech are among US companies evaluating enzyme-linked immunosorbent assay (ELISA) tests that incorporate recombinant antigen, which avoids at least some of the problems caused by the natural variability of the virus.

Some preliminary data on the new tests were given last week at a government-sponsored conference at the National Institutes of Health. In addition, Abbott claims to have a prototype test that detects viral antigens in the blood, as distinct from the human antibodies that existing ELISA tests look for. A workable antigen test would be a much surer guide to those at risk of developing the disease or to carriers of the disease; it is, however, technically more demanding because antigens are present in much lower concentrations than antibodies.

The existing licensed tests on the UK market, supplied by Abbott, Electronucleonics and Litton, are generally agreed to have effectively protected blood banks; virtually all blood samples from AIDS patients are detected. But there remains a substantial number of repeatedly reactive samples that do not show evidence of antibodies on a Western blot test, even when technical errors are excluded.

In Western blotting, the principle of the better-known Southern and Northern blotting techniques is extended to the de-

tection of proteins rather than nucleic acid fragments. The protein mixture for assay is size fractionated by gel electrophoresis, followed by transfer to a support in such a way as to preserve the relative positions of the separated proteins. Specific proteins attached to the support can then be detected with antibodies.

The Western blot is considered more reliable than ELISA testing, although even so it shows appreciable variation between laboratories, according to Carl Saxinger of the National Cancer Institute. One reason for the large number of false positives is mutation in the virus populations that supply the antigens used in tests. Recombinant antigens would be more stable (although the problem of sample virus variability would remain) and so perhaps reduce the false-positive rate. A cocktail of different recombinant antigens might be the best solution. Recombinant antigens have the additional advantage of avoiding the need to culture live virus.

The false-positive problem has led to harrowing decisions about what to tell patients whose samples appear positive, although manufacturers stress that current tests are not intended for use in diagnosis. The presence of antibodies, even if confirmed, does not necessarily imply that overt disease will develop. Looking to the future, some manufacturers expect to be producing batteries of confirmatory tests for antibodies characteristic of different stages of infection and disease. Together with a viral antigen test, these may allow immune people to be recognized and thus provide useful data on the progress of infection. **Tim Beardsley**

British forests

Tax breaks for broadleaved trees

MULTIPURPOSE use is an increasingly popular catchword among natural resource managers, implying that seemingly conflicting demands on a resource, such as a trout stream and a hydropower dam, can in reality be harmonized. The concept is the lynchpin of the British Forestry Commission's (FC) new broadleaved woodlands policy, intended to give enough tax relief and grant aid to make growing broadleaved trees profitable. These incentives, according to the commission, will persuade landowners voluntarily to manage now-neglected broadleaved woodlands and also to plant new stands, thus providing a habitat for native British wildlife and soothing the British eye for beauty, which prefers a native oak to an exotic conifer.

The principal criticism of the new programme, from foresters and conservation groups alike, is that broadleaves are hard to grow and that the commission's system of advisers for helping landowners will be understaffed and underbudgeted. The commission, on the other hand, claims the government is committed to the advisory system and will review it in 3–5 years.

One-third of Britain's productive timberland (566,000 hectares) is planted in broadleaves, with virtually all of that in the lowlands and in private hands. Broadleaves management, therefore, is clearly a question of motivating the private sector. Neither broadleaves nor conifers are self-supporting timber industries, according to Mr David Conder of the Council for the Protection of Rural England; therefore, broadleaves, for which there is "an environmental and recreational preference", should be given the more attractive tax break.

Conifers and broadleaves, however, are more dissimilar than Mr Conder suggests: the latter grow more slowly, need more room and produce less usable timber. Mr Esmond Harris of the Royal Forestry Society of England and Wales claims, for example, that Corsican pine stands in the Forest of Dean are three to four times more valuable than the broadleaves grown there.

Private landowners would like to see a profit within their own lifetime, so research by the Forestry Commission and private interests into higher-quality and faster-growing broadleaves is as important as new tax and grant schemes. Mr Harris points to plastic tubes that shelter young broadleaves, helping them grow four times faster than by conventional methods and halving the 120–150 years many broadleaves need to mature. Making broadleaves a commercial success, says Mr Harris, is the key for conservation efforts: wildlife needs can then be incorporated into productive woodlands rather than forcing land to be set aside just

for habitat.

Part of the growing public interest in broadleaves is a backlash against conifers, which it is claimed do not support ancient woodland plants, create acidity on the soil's surface and leach nutrients from the soil. Mr Mark Anderson, forest ecologist at the commission, says conifers do not deserve such blackballing. He points to the cycle of canopy closure and species differences in litter properties — whether the leaves pack down and rot or not — as the crucial elements in plant colonization. Conifers do acidify surface soil, particularly in the uplands where the soil has little buffering capacity, but these properties peak and trough and then revert to approximately their initial values by the end of the rotation. And finally, tannin does

wash out of conifer leaves, chemically associate with soil nutrients and wash to lower levels; but this behaviour happens primarily in poor soil and is also associated with broadleaves.

The upland conifer issue, according to Mr John Andrews of the Royal Society for the Protection of Birds, comes down to a conflict between agriculture and forestry. As agriculture has been allowed more hill land, forest plantations have been pushed further north and west into poorer and poorer land that is also valuable bird habitat. There the trees do not grow large and are subject to considerable windfall, which means there are no old trees for birds to drill holes in. By "moving the centre of gravity of forestry downhill", says Mr Andrews, the Forestry Commission could grow better trees while leaving the windswept moors with the poorest soils to the birds and animals that do in fact thrive there.

Elizabeth Collins

Deep-sea clams

New find near Japan's coast

Tokyo

GIANT clam colonies, first reported from the deep sea off Japan only two months ago (*Nature* 315, 624; 1985) are now popping up right, left and centre, from as little as a kilometre off the Izu Peninsula to a depth of over 5,000 metres in the Japan Trench. Discovery of the colonies provides a golden opportunity to study these strange organisms, believed to draw their energy from a source in the Earth beneath them rather than the Sun above.

Days after the French submersible *Nautille* located giant clam colonies at a record depth of 3,840 m in the mouth of the Tenryu Canyon off the Pacific coast, Japan's submersible *Shinkai 2000* found similar colonies in Sagami Bay at a depth of 1,300 m, a mere stone's throw away from the hot spring resort town of Ito on the Izu Peninsula. A month later, *Nautille* shattered its own record with the discovery of another clam colony at 5,640 m on the steep inner wall of the Japan Trench, immediately opposite the First Kashima seamount.

This discovery was made by Kantaro Fujioka of the Ocean Research Institute, University of Tokyo, during the second leg of the Franco-Japanese project Kaiko, led jointly by Guy Pautot of IFREMER and Kazuaki Nakamura of the Earthquake Research Centre, University of Tokyo. The colony, measuring two by one metres, consists of banana-shaped clams up to about 15 cm in length which have tentatively been identified as *Calyptogena* sp.; they are stacked together forming an imbrication parallel to the long axis of the colony.

As in the case of the Tenryu Canyon colonies, a positive temperature anomaly of about 0.3°C was associated with the colony which occurred in a slight depress-

ion. There are reports that the French have found an even deeper colony during the third and final leg of Kaiko now in progress at the northern end of the Japan Trench.

The colonies off the Izu Peninsula are larger, the biggest living colony being twenty by five metres, and, like the Tenryu Canyon and Japan Trench colonies, are associated with thrust faulting caused by subduction as the Izu Peninsula riding on the Philippine plate ploughs into the rest of Japan. Clams collected by Teruaki Ishi of the Ocean Research Institute have been identified as *Calyptogena soyoe* by Sugura Ohta of the same institute, who also recognized in video records what appear to be vestimentiferan tube worms attached to a ledge above the clam colony.

Unfortunately, *Shinkai 2000* was unable to collect specimens of the worms, which have not been found at the deeper colonies. Despite proximity to hot springs on land, Dr Ohta does not think the colonies off Izu derive their energy from hot vents as at mid-ocean spreading ridges but, rather, suspects that they are sustained by old seawater "squeezed" out along fault planes by subduction.

The find off Izu is well within the 2,000-m depth range of *Shinkai 2000* and lies very close to land, opening up the possibility of regular *in situ* experiments. A deeper-going Japanese submersible capable of examining the Japan Trench colonies is at the design stage. Although the new submersible was originally intended to have a depth limit of 6,000 metres as in the case of *Nautille*, an advisory body to the Science and Technology Agency recommended last month that this should be increased to 6,500 metres, thereby allowing access to all but the deepest trench basins off Japan.

David Swinbanks

US vaccination

Statutory compensation urged

Washington

THE Institute for Medicine last week added its voice to the many others complaining that present disincentives to the development and production of vaccines constitute a threat to public health in the United States. There is no knowing whether this latest warning will carry weight with the administration, the Congress and the courts.

Plaintively, the committee responsible for last week's report, *Vaccine supply and innovation*, remarks that recommendations of previous committees have been ignored "for reasons unrelated to the potential utility of the recommendations", which is a polite way of saying that political considerations and/or idleness have supervened.

The Institute of Medicine's committee, under its chairman Jay P. Sanford of the Uniformed Services University of the Health Sciences, argues that continuing immunization against an extended range of infections should be an essential part of public health provision in the United States. But the committee says that vaccine supply is made "precarious" by the obstacles faced by manufacturers, some of which are economic (attenuated patent protection, for example) and others legal, particularly the uncertainty about liability for injuries associated with the administration of vaccines.

The most arresting part of the commit-

tee's argument is its careful demonstration that the application of the present law is shot through with anomalies. Its chief recommendations are that there should be a national vaccine commission in the United States to anticipate problems connected with vaccination, together with a statutory compensation scheme.

Paediatric vaccines are the most immediate worry, according to the committee, which says that two of the three manufacturers of DTP vaccine (offering protection against diphtheria, tetanus and pertussis or whooping-cough infections) had given up manufacture of the vaccine a year ago. Since then, apparently, Wyeth has subcontracted to supply Lederle with vaccine not for distribution under its own label, while Connaught Laboratories (a subsidiary of Squibb) has managed to obtain liability insurance to allow manufacture to be resumed.

There are similar problems with the supply of poliomyelitis and the combined measles, mumps and rubella (german measles) vaccines, with stockpiles held by the Centers for Disease Control down to 15 and 12 weeks respectively, partly because of congressional niggardliness on the cost of the stockpile programme. The committee says that the supply position is "disconcertingly unstable".

On the economic front, the committee says that part of the reason for the twofold reduction of the number of manufacturers

making vaccines between 1968 and 1975 may be that the vaccine business is less profitable than the development of pharmaceuticals. The cost of developing new vaccines is estimated at between \$20 million and \$30 million, shared roughly equally between manufacturers (who pay for clinical trials and production development) and the federal agencies, which bear the cost of basic research.

The total value of the vaccine market in 1982, on the other hand, is estimated to have been \$170 million, which the committee says may be only a fraction of the sales in a single year of a successful drug. Even so, according to the committee, the economic basis of the vaccine industry may recently have been strengthened by last year's legislation extending the patent life of new medicines to compensate for licensing delays, the decision of the Supreme Court in 1980 that strains of living organisms may be patented and administrative decisions during the past few years that have given universities and other recipients of federal grants the right (and even the responsibility) to exploit intellectual property arising from their research.

On the legal front, the committee is less optimistic. A survey among manufacturers in the spring last year showed that there were 166 outstanding liability suits, and a further 65 were laid in the following twelve months. Only a small proportion of the outstanding claims had been settled in the interval, often for amounts of the order of \$1 million. Some manufacturers claimed that legal costs of defending vaccine suits amounted to "several millions of dollars a year" and that the cost of defending a single suit might amount to \$500,000.

Citing the law as it stands, the committee says that a manufacturer cannot be held responsible for damage done by a vaccine which is not defective, but goes on to complain that courts in various of the United States have been prone to interpret the legal position more broadly, and in unpredictable ways.

In legal suits during the past fifteen years, it seems that a manufacturer's duty to warn recipients of a vaccine (or, when children, their parents) of the dangers of vaccination has commonly contributed to the award of damages against manufacturers. The committee cites one suit in which the manufacturer was held liable for the occurrence of polio in the parent of a child vaccinated with Sabin (attenuated live virus) vaccine and administered by the Texas Department of Public Health. The courts held that the parent should have been told that the prior administration of Salk vaccine would have avoided the risk; the Institute of Medicine's committee points out that Salk vaccine was not being manufactured at the time.

In another case, a manufacturer was found to be liable for damage caused by Sabin vaccine because the physician who had administered it privately felt that the warning on the package insert that a few

A check-list of vaccine hazards

THE Institute of Medicine report says that attempts to measure the risks associated with vaccines are complicated by the difficulty of relating cause and effect, by the circumstances that vaccine injury tends to be regarded seriously only after the incidence of damage from the uncontrolled infection has been substantially reduced and because of the lack of knowledge of the mechanism of vaccine injury. But immunodeficiency argues against the use of vaccines of any kind.

On the basis of experience in the United States, the report gives the following data. Pertussis. The frequency of fever (39°C or more) is roughly 7 per cent within 24 hours of vaccination, apparently a consequence of the pertussis component of DTP (diphtheria-tetanus-pertussis) vaccine. The frequency of permanent injury, in the form of encephalopathy, is reckoned, from British data, to be one in about 150,000 in infants given three doses in the first year of life.

Diphtheria. While earlier preparations of the toxin produced temporary reactions, better purification has eliminated fatal or disabling reactions.

Tetanus. Anaphylactic reactions to tetanus

toxin are recognized at the rate of 1 per 1.5 to 2.0 million doses, but no fatalities are known.

Poliomyelitis. The risk of contracting paralytic polio from its oral vaccine is estimated at 1 in 11 million doses. But the committee says that consideration is now being given to switching back to inactivated polio vaccine.

Measles. Between 5 and 15 per cent of infants at 15 months develop a fever of 39.4°C a week after vaccination. Measles encephalitis, a complication of the natural infection, is estimated permanently to affect fewer than 1 in a million vaccinated children. The rate of slow-virus infection, leading to progressive fatal neurological disease, is too small to be estimated.

Rubella. Persistent arthritic conditions are recognized in a few vaccinated women.

Mumps. The report recognizes no permanent consequences of the use of mumps vaccine except that of allergic reaction to proteins from the eggs in which the virus is grown.

Influenza. The 500 cases of Guillain-Barré syndrome reported after the use of swine flu vaccine in 1976 are "unprecedented"; the cause remains unknown. □

vaccinations in a million might lead to poliomyelitis infection was not sufficiently explicit to pass on to the parents of a vaccinated child. The committee says that "the sceptical reader" may fairly conclude that US courts behave as if "the manufacturer is responsible for all damages caused by a vaccine".

The great swine influenza fuss of 1976 is offered as a complicating precedent. Out of fear of a swine flu epidemic on the scale of the 1919 influenza epidemic, and in the face of manufacturers' unwillingness to produce vaccine in the time required without indemnity, the US Congress passed legislation placing liability for damage on the federal government, which was afterwards interpreted (by the then Secretary of the Department of Health Education and Welfare, Joseph A. Califano) to imply that strict liability for all injuries would rest against the United States.

In the event, the influenza epidemic did not materialize, but among those vaccinated, between 1 in 100,000 and 1 in 200,000 developed the neurological complications of the Guillain-Barré syndrome. The US government has already paid out \$73 million to claimants. Legal cases stemming from the episode have tended to be settled on the basis of strict liability.

The Sanford committee's proposal that there should be a permanent vaccine commission takes the somewhat unusual form of recommending that the commission should be set up, at a cost estimated at \$1 million a year, as a congressionally chartered body, reporting annually. But the committee says that other locations of the commission within the US government structure would be acceptable.

The committee argues that the case for compensating victims and their families stems from the fact that vaccination is sometimes compulsory (as for children entering the educational system) and that vaccination of an individual contributes to the public as well as the personal good.

On balance, the committee is for a system in which compensation would be paid from a fund recruited by a levy on vaccine sales. It emphasizes that such a scheme would not absolve manufacturers from the duty to avoid defects in their products.

The amount of compensation to be paid, the committee argues, should be devised so as to "restore the incentive to participate" in vaccination programmes, avoid financial devastation of victims' families, keep vaccine costs low, avoid disproportionate spending on a field of public health which is "important [but] not the only legitimate claim on . . . resources" and to remove the incentive to litigation.

The committee leaves to others the question of whether a compensation scheme should be accompanied by an extinction of people's right to sue under the law of tort, but instead offers a menu of ten alternative schemes from which readers may choose □

Australian space

Never too late to change

Canberra

WITH the launching of the first three domestic communications satellites of the Aussat-1 series later this month, the Australian government faces some difficult policy choices. The Minister for Communications, Mr Duffy, has warned that changes may be in the offing to regulations that have been on the books for 30 years. Deciding how the local broadcasting market is to be carved up between the large national networks and the smaller regional broadcasters is one problem; the provision of telephone services to remote outback communities another. While Telecom, the government's monopoly carrier for public switched telecommunications, is protected in its role by the Satellite Communications Act 1984, Aussat will compete in the areas of private leased networks, data communications and in other fields. For Australia's space-related industries, however, the big question is whether the government will assist them in rekindling an earlier vigour which petered out when ELDO (European Launcher Development Organization, of which Australia was the launch-site member) gave way to ESA, the European Space Agency, leaving the locals without a space policy coordinating body.

At a time when national communications satellites are going aloft all round the world, Australia's share in the manufacture of space systems has dwindled to the point where the weather map appearing nightly on Australian television screens is beamed down from Japan's GMS-3 meteorological satellite and people are beginning to wonder whether the governments of countries with more sophisticated space technologies know more about Australia's natural resources than do Australians.

Whether all this changes will depend to a great extent on the government's response to the recommendations of the Madigan report — a space policy for Australia published by the Australian Academy of Technological Sciences which asserts that Australia still possesses the technological and industrial ability to develop an effective space programme, but that the actors will need to be welded together by prompt government action in the formation of a space technology and research authority.

Furthermore, the government will have to invest about \$A100 million over the next five years in order to stimulate academic space research and to put Australian companies in a position to bid for international contracts by first weaning them on national research and development projects.

The Madigan report also recommended that the Australian Landsat facilities be

upgraded, and identified equipment for ground receiving stations and the remote sensing of Earth resources as immediate priorities for Australian industry.

The Commonwealth Scientific and Industrial Research Organisation (CSIRO) was quick off the mark. Having both established a space science study group and designated space as one of its principal growth areas, CSIRO set up a body to coordinate the organization's efforts in the field, called the CSIRO Office for Space Science and Applications (COSSA). It is headed by Dr Ken McCracken, formerly chief of the CSIRO division of mineral physics.

As soon as the Madigan report was published, COSSA put forward a detailed proposal of the steps that would be needed if Australian industry was to reach the point where it could gain the prime contracts for Aussat-3, due to be built in 1994.

Two current projects were pointed out as good examples of the kind of work which would qualify Australia as a contractor: the scanning infrared radiometer developed at the CSIRO division of atmospheric research (capable of detecting 0.3° temperature changes in the surface of the sea) and the large-format detector of the Mt Stromlo-based Australian part of the FUSE/Lyman (far ultraviolet space explorer) satellite.

But the timing is crucial. COSSA estimates that local industry may have gained sufficient experience in spacecraft design to play a considerable part in the design phase of Aussat-2. But the same cannot be said for the 1989 construction phase. The bridging of this "experience gap", COSSA suggests, should be Australia's participation in the refurbishing of the US Spartan reusable spacecraft and the construction of the scientific instrument package to fly on it.

The resulting satellite — to be launched in 1988 and called Mirrabooka, an aboriginal word for the Southern Cross — will be a fast-turn-around vehicle for further experiments and, if all goes well, may qualify Australian contractors for about 30 per cent of the space acquisitions of Aussat-2, due for launching in 1992.

The government has already taken notice. Last week, the Minister for Industry, Technology and Commerce, Senator John Button, announced the award of a \$A3 million contract for three 18-m Earth stations for international television transmission of the America's Cup defence, a \$A2.5 million contract to develop rooftop Earth stations for the Intelsat business services network and a \$A18 million contract to build seven 22-m antennas at Culgoora and Siding Spring for the Australia Telescope (see *Nature* 311, 500, 1984).
Jeffrey Sellar

Whaling

Inaccuracies in numbers

THE difficulty of counting whales accurately has muddled the already turbulent management task of the International Whaling Commission (IWC), whose recent meeting in Bournemouth, Dorset, was the last before a ten-year moratorium on commercial whaling is due to take effect in 1986. The Scientific Committee of IWC will spend that decade on a specific task: researching ways of counting whales and analysing how to incorporate these numbers and their uncertainties into the



management procedure.

Counting whales is not as easy as counting trees or fish. Scientists are confident that the computer models and counting methods used to extrapolate population size and growth trends for trees or fish are accurate — or at least accurate enough to manage the resource. Trees have the comforting habit of standing still, and we know the biology and reproductive rates of fish well enough to predict trends. There is increasing recognition, however, that similar methods applied to whales result in confidence limits so wide that only an extreme change in numbers will register.

Whaling data have traditionally been garnered from the whaling industry itself. A common method has been that of "catch per unit effort", which derived from fisheries work. An index is established from the size of the catch and the amount of effort involved: in other words, if the same effort is involved and a catch is half its former size, the population is half its former size. Sidney Holt, a fisheries biologist now acting as a consultant on marine affairs, is one of many scientists who point to the difficulty of quantifying "effort" and the nonlinear relationship of the index to the actual population size.

Another option is to mark and recapture. Whales are either marked artificially or recognized by their natural marks. If 100 whales are marked or recognized and the next year five of them are recognized again or caught by whalers, the actual population is approximately 20×100 in size. Individual humpback and right whales, according to Dr Lex Hiby of the Sea Mammal Research Unit, which is supported by the Natural Environment Research Council, are easy to recognize and

blue whales are potentially so because of a lacy pattern on their skin; these species, however, have already been decimated to the point of being protected species. And IWC is having to recognize that artificial marks do not work well for the commercially viable minke and sperm whales. The metal bolts used can kill the whales as they are marked, there is no way to know if a bolt has actually lodged in a whale, and it is now being shown that the bolts are sometimes extruded.

The sightings method, criss-crossing a given area by plane or ship and estimating the density of whales, is now recognized as offering the best possibilities for an accurate counting model. As at present used, the confidence limits of this method are very wide, says Holt — so wide that a population could be a third of its original size before the change was recognized. The problem is that the method is based on one developed for tree research, which assumes that if a tree is right on the observer's path, it will be seen. Whales, however, dive and travel towards and away from the observer, which means there is no assurance that whales right on the path will be counted accurately. The Sea Mammal Research Unit is part of an international effort to control variability

in sightings counts, running such experiments in the Antarctic as counting whales from ships moving in parallel or counting whale blows per unit area per unit time.

The scientific committee's review of the data gathered during the moratorium, as well as of research priorities and survey methods, is intended to establish more accurate guidelines for gauging the impact of whaling. If the effort fails, says Dr John Harwood, head of the Sea Mammal Research Unit, it will be a "sad reflection on science and management". It will also in part determine whether Mr John MacGregor, Minister of Agriculture, Fisheries and Food, was right in calling the moratorium "a landmark in the long history of man's relationship with these most fascinating creatures of the sea".

But whether there really will be a lapse in commercial whaling depends on such factors as US pressure on Japan and whether South Korea and Iceland do in fact kill over 200 whales each year for "scientific research". Even if IWC had real teeth and enough money, the world's oceans are such a huge area to monitor that the success of the moratorium is dependent on the cooperation of the countries that have the most to gain by ignoring it. Luckily some species of whale, notably the blue, will receive added protection under an international convention to protect threatened migratory animals, which Britain signed last week. **Elizabeth Collins**

French research

Southern festival of science

WHY not take in a laboratory or two on the way south to the Côte d'Azur? Until 18 August, the leading French research council (the Centre National de la Recherche Scientifique) is running a kind of continuous science festival at three of its laboratories in the south of France. The project is the successor to a major CNRS achievement last year, when it took over 2,000 square metres of space under the Eiffel Tower in Paris for 2 weeks for an exhibition on all aspects of communication, from technology to languages and involving journalists, actors and a variety of forms of presentation. Some 10,000 visitors attended, and M. Goéry Delacotte, CNRS director of information, is hoping for a similar success in the south.

"People are always asking us what we have discovered with their money", says Delacotte, and with these exhibitions CNRS is setting out to reply — and, no doubt, to win friends and influence people. It has set up a full-time "exploration workshop" in Meudon, near Paris, with a model-making staff of 10. Some of the results are in use at the new festival in the south.

Delacotte is particularly proud of a lens designed to show the precise gravitational lensing of a quasar by a nearby galaxy, separating the quasar light into three

different images. That is on display at the 13-telescope Observatoire de Haute Provence, near St Michel. Also on show are atmospheric studies by LIDAR, work in interacting galaxies (the Magellanic Clouds) and studies of Halley's comet (in which the observatory is closely involved), all with mock-ups and exhibits.

Other sites involved are the solar energy laboratory at Odeilla-Font-Romeu (in the east Pyrenees) and a group of marine laboratories at Villefranche-sur-mer. At all these exhibition sites there are regular lectures, exhibitions and activities for children.

What is more, visitors will also find real scientists of whom to ask questions, it now being an official duty of a French scientist, and one which can in principle advance his or her career, to popularize science.

CNRS is spending some FF 2 million (£160,000) per site for the festival, Delacotte estimates. If this year's trial is successful, the exercise may spread to cover the whole of France. "It's important to reach a critical mass", Delacotte says.

Robert Walgate

For more information on addresses and opening times, contact the Direction de l'Information Scientifique et Technique, CNRS, 15 quai Anatole France, 75700 Paris, or telephone Paris 555 9225

Nuclear winter

Canadian forest burn as model

Washington

US and Canadian scientists flew to northern Ontario last weekend to watch what happens when 6 square kilometres of dry forest is doused with a petroleum mixture and set alight. Their aim was to see whether measurements from such planned fires could help to decide how likely it is that a nuclear war would give rise to "nuclear winter" — a global lowering of temperature caused by soot in the atmosphere. But the fire has already caused some unplanned political fallout.

The forest, 25 kilometres north-west of Chapleau, was infested with spruce budworm and was due to have been burned anyway — the Ontario Ministry of Natural Resources organizes about 50 such "prescribed burns" every year. This one, according to Bob Thomas of the Aviation

and Fire Management Center in Sault Ste Marie, was no different from any other, though it was one of the larger ones. The forest, which had been bulldozed flat, was ignited from several points by helicopters flying in spirals, drenching the wood with a thickened petroleum compound much like napalm. This ignition technique, developed only last year, means that a forest can be burned very quickly.

Mike Frankel of the US Defense Nuclear Agency said last week that the purpose of the visit was simply to see whether the fire would be a true mass fire of the sort produced by nuclear explosions, as distinct from one composed of moving fronts, like a natural forest fire. If the fire were sufficiently intense, the trip might lead to a formal collaboration with the Canadians, with detailed observations of future prescribed burns from aircraft.

Although burning wood produces much less sooty smoke than would a burning city, and is therefore less likely to have climatic effects, there is still much that can be learned from studying mass forest fires, according to Frankel: the relation between energy release rates and the height of the convective column, the fate of entrained water vapour and the optical properties of the smoke plume as it is dispersed are all effects that cannot be predicted with confidence by extrapolating from small fires.

Canada has no formal nuclear winter research programme, although a report earlier this year from the Canadian Royal Society recommended that Canada should contribute to studies of this phenomenon. The idea of studying prescribed burns arose out of conversations between Frankel, George Carrier of Harvard University, who chaired the US National Academy of Sciences study of nuclear winter, and Brian Stocks of the Canadian Forestry Service. The fire was also watched by university researchers and by representatives of Environment Canada and the Canadian Department of National Defence; from the United States came observers from the Defense Nuclear Agency, TRW Inc. and Los Alamos National Laboratory. Any future collaboration would involve, on the US side, the Defense Nuclear Agency, which is (together with Lawrence Livermore National Laboratory) the main agency studying nuclear winter; the total US nuclear winter budget is put at \$5.5 million.

Nuclear weapons are a politically touchy issue in Canada and politics might limit the extent of future work. Although last weekend's observation was originally to have been an informal affair, indignant local press coverage obliged the Canadian authorities to be seen to be in charge, which they did by placing a limit on the number of observers.

Tim Beardsley

Athletic doping

Hungarian owns up

A HUNGARIAN pharmacologist, Dr Zoltan Torma, has admitted administering banned drugs and anabolic steroids to Hungarian athletes, the first time that such an admission has been made in the socialist bloc. Writing in the Budapest literary weekly, *Elet és Irodalom*, Dr Torma, who is employed at the National Physical Education and Sports Centre in Budapest, justified his actions by claiming that, left to themselves, the athletes would endanger their health by taking arbitrary doses. A qualified practitioner, he said, can at least administer the minimum necessary.

Dr Torma's article has sparked off an extensive discussion in the media on doping. Hungary's National Sports Office has in recent years spent the equivalent of US\$250,000 on anti-doping measures, but there seems to be tacit acceptance among Hungarian athletes and sports coaches that, without doping, Hungarian athletes cannot hope to compete internationally against competitors who resort to unauthorized use of drugs.

Dr Torma's excuse is not new. It was offered, for example, some years ago in the United States, in defence of allegations of doping in the case of the San Diego Chargers. What is significant, however, is that even the chief doctor of the Sports Office, Dr Istvan Kojtar, openly admitted, in an article in the sporting daily *Nepsport*, that it is virtually impossible to stop an athlete taking drugs if he is really set on doing so, and that doctors who refuse their assistance know that athletes risk severe damage to their health by self-administration. He suggested, however, that there should be a greater "social investigation" into the use of drugs in sport that would place it within the context of other social problems such as "non-sporting" drug abuse.

Dr Torma clearly agrees. He called for a clean up of Hungarian domestic sport, in which officials, coaches and athletes would be obliged to take a clear-cut stance against doping and the misuse of anabolic steroids. This, if Hungarian athletes' suspicions of their international competitors are correct, could considerably weaken Hungary's chances in international competition for some years.

The answer, clearly, is better doping control at the international level. Dr Torma himself would like to see a situation in which the International Olympic Committee would have the right to enforce random doping tests on any of its members at any time, so that athletes could be checked during training, and not just, as at present, immediately before a competition. However, not all members of the committee support this idea.

Vera Rich

Aleksandrov still not found

THE fate of Dr Vladimir Aleksandrov, the Soviet expert on nuclear winter who disappeared from an international meeting in Spain last March (see *Nature* 4 July, p.3), continues to cause concern to his colleagues abroad. Requests for information to Soviet colleagues have evoked only noncommittal responses such as "It was a tragedy, but we do not have many details". Some Soviet scientists have stated that their government has made requests for information through "diplomatic and other channels", but without success. According to one source, the Soviet government has also asked the International Committee for the Red Cross (IRC) to trace Aleksandrov, but Pascal Gondrand, spokesman of the IRC information department in Geneva, has no knowledge of any such request.

Although it has still not proved possible to establish Aleksandrov's movements immediately before his disappearance, details of his family situation received in the past month suggest that it is extremely unlikely that he disappeared voluntarily. He was known to be seriously concerned about his wife's health, and had sought the help of a London specialist, to whom he had sent, through a colleague, the results of a liver biopsy on Mrs Aleksandrova, carried out in the Soviet Union. The specialist had replied that it was impossible to complete a diagnosis solely on the basis of the materials so far supplied. Aleksandrov, however, was due to take part in a meeting of the ENUWAR (Environmental Consequences of Nuclear War) study group at the University of Essex in June, and (until he vanished) it was understood that he would take advantage of the meeting in England to deliver further diagnostic material to the London consultant.

Vera Rich

The meaning of "human life"

SIR—In the context of the recent, abortive Unborn Children (Protection) Bill in the UK House of Commons, opinions were voiced about the propriety or otherwise of conducting experiments on human embryos which were "alive"; but I am aware of no serious attempts by biologists to clarify the issue by providing an authoritative definition of what constitutes human life observationally.

Since this issue is likely to recur, I should like to summarize the meaning of (human) life as known from observation.

(1) The property of life, whether human or otherwise, is observably of two different qualities: namely, (a) dependently viable, protoplasmic life (at the level of scale of the molecule), and (b) independently viable, organismal life (at the level of scale of the individual), which in humans is also called "spirit".

(2) Dependently viable, protoplasmic life (1a above) — which, to the best of our knowledge, originated some 3,000 million years ago, and has continued (by means of repeated cell division) without interruption to the present day — characterizes the germ-lines of human beings: that is, the succession of gametes, both ova and sperm; which mediate human inheritance.

(3) Independently viable, organismal life or spirit (1b above) — which observably originates at viable birth, and continues without interruption until organismal death — characterizes the lives of human beings as individuals.

(4) It is thus not sufficient to refer simply to human "life". It is necessary to specify that particular quality of life to which reference is to be made: that is, whether to (a) continuous, protoplasmic life: which generally lingers in body tissues, even after organismal death, and which (as when a body is artificially ventilated) may continue so to linger indefinitely: or to (b) discontinuous, organismal life or spirit, which organismal death invariably displaces.

(5) How, then, shall we specify the quality of life in an embryo (or a fetus) *in utero*?

(6) That it possesses the quality of dependent, protoplasmic life (1a) follows from its observable intra-uterine growth (by cell division) and development (by cell differentiation).

(7) That it does not possess the quality of independent organismal life (1b) before viable birth, follows by definition.

(8) That it does not necessarily possess the quality of potential organismal life, follows from the finite probability of either unavoidable miscarriage or unavoidable still-birth (and, perhaps, as a never-viable monster).

(9) It follows that the life quality which is necessarily attributable to an embryo or fetus *in utero* is that of protoplasmic life: treated as an extension of the

protoplasmic life of its mother to which (for its supply of energy) it is dependently attached, and until such time as, by virtue of its viable birth, a protoplasmically alive fetus acquires the organismal quality of an independently alive human being.

(10) That is to say, human life passes through the following general cycle: (a) *In utero*, the embryo/fetus is alive protoplasmically, being dependent (for its energy) on its mother. (b) After viable birth, the human individual is alive both protoplasmically and organismally, being dependent (for its energy) only on itself — that is, it is independent. (c) After organismal ("brain") death, the body of the human individual remains alive protoplasmically, but no longer independently. Therefore, *unless* it be quickly provided with a surrogate mother in the form of an artificial ventilator, inevitably the organismally dead human being progresses also to protoplasmic death.

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Down with metric

SIR—Your anonymous commentator (*Nature* 27 June, p.702) is both factually and conceptually incorrect. The first space shuttle laser-stabilization test did not fail because of confusion between nautical and statute miles. The ground-based laser was on a 10,023-foot high Hawaiian volcano. The on-board computer was given this height in feet rather than the nautical miles it was expecting. The volcano became in the computer's mind a 10,023-mile-high mountain. Since the peak would be above the shuttle, the on-board computer faithfully rolled the shuttle so that the mirrored side faced upward to receive the anticipated light-beam. When the on-board computer was later informed that the height was really in feet, the system worked perfectly.

Your commentator then runs on with unfounded remarks about alleged confusions in the English unit system. But there is no confusion. Practically everyone carries at all times two reasonably accurate standard feet, and miles are what it takes a thousand paces to cover. Arguments that the English system is "unscientific" collapse when it is realized that much of that system is based on binary arithmetic (64 pints per bushel, 1/32 in., etc.) or the duodecimal system, both of which possess distinct mathematical advantages. It is disheartening to see metrification pleas emanating from the same nation that gave us Lord Kelvin's absolute foot-grain-second (fgs) electromagnetic units, a system I find quite handy.

Spurious appeals to the French Revolution will not do. Their ten-day week and similar inanities never caught on. The

metric measurement was at first voluntary in France since it was assumed that "logic" would prevail. In fact, logic did prevail — people simply ignored the fool units until the powers-that-be made them compulsory in 1840. Things have not changed. Only where the crushing weights of state power bears down on a helpless citizenry does the metric system prevail. Perhaps it is time for a counter-revolution.

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NERC and unions

SIR—Your report of Professor E.R. Oxburgh's Royal Society paper, on UK geophysics (*Nature* 27 June, p. 709) may have given readers a false impression of trade union attitudes within the Natural Environment Research Council (NERC).

Oxburgh's fears that NERC's commercial competitiveness is constrained by "labour legislation and trade union agreements" are unfounded. All employers are, or course, bound by legislation and the parlous state of NERC's finances can hardly be sought in this direction. The NERC trade unions may be forgiven for feeling slightly flattered that Professor Oxburgh thinks us able to compel our management on the topic of short-term contracts of employment. The truth is, however, that present agreements on so-called "period appointments" were reached after much disquiet had been expressed both by staff and management.

Management was concerned that people working on short-term projects spent one year reading around the topic, one year working upon it and their final year looking for another post. The NERC trade unions agreed that this was an inefficient way of deploying our resources and were naturally concerned at the lack of job security and pension rights. It was against this background that a code of practice was agreed.

Whilst chairman, Sir Hermann Bondi consistently defended present practices. Far from compelling the British Geological Survey to take short-term staff onto the permanent complement, trade unions have often protested to management when such action has been taken summarily and without following due procedures allowing for full competition for filling vacant posts.

Trade unionists within NERC are fully committed to working for an efficient and vital organization. It is a discredit to the scientists and support staff who make up those unions to cast them as obstacles to the realization of NERC's potential.

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Ferroelasticity comes of age

Crystals that can be made to twin under the influence of mechanical stress have been known for some time; neodymium pentaphosphate may become the basis of novel optical and acoustic devices.

WHAT is the object shown in the accompanying photograph? An optical grating perhaps? Then something has gone wrong with the ruling engine, for the rulings are not uniform in width but wedge-like, making a zig-zag pattern across each strip of the object. Yet potentially, the first guess is correct.

What the photograph shows is the pattern of transmission of polarized light through a single crystal of neodymium pentaphosphate (NPP) prepared by a technique described by Stephen Meeks and B.A. Auld in the 15 July issue of *Applied Physics Letters* (47, 102; 1985). The width of the periodic bands ranges from roughly $18\text{ }\mu\text{m}$ to $6\text{ }\mu\text{m}$ (on the right).

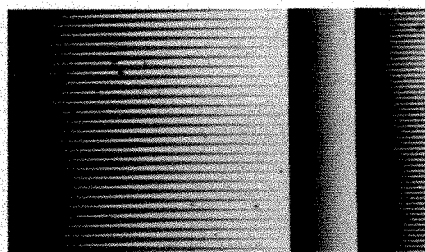
What excites Meeks and Auld is that they have a technique by which the spacing of the grating elements can be systematically changed, or tuned, at least for spacings greater than $0.5\text{ }\mu\text{m}$. Since NPP is also a laser material, and one with an exceptionally low threshold because of the exceptional fluorescence of the material, they hope to be able to have a laser with a built-in optical grating which can, moreover, be instantaneously tuned. But the material has other obvious applications in, for example, the construction of acoustic gratings and filters.

This curious development has been on the cards for the best part of a decade, since the recognition that the pentaphosphates of the rare earth elements (but also those of lead and bismuth) are capable of forming crystals that are ferroelastic, which is merely a way of saying that they may be induced to change their microscopic state by means of macroscopic elastic stress. Indeed, NPP crystals, which tend to be produced as thin plates that may be up to a centimetre long, can be flipped from one stable state to another by forces that seem quite trivial; stroking the surface of a crystal with a toothpick seems to suffice.

The explanation of this peculiar behaviour of NPP, and of materials like it, seems first to have been given by H.P. Weber, working at Bell Laboratories in the 1970s (see, for example, Weber, H.P., Tofield, B.C. & Liao, P.F. *Phys. Rev. B*11, 1152; 1975). The essence of the explanation is that NPP may crystallize in the form of monoclinic crystals which differ hardly at all from the higher orthorhombic symmetry. Indeed, X-ray diffraction shows NPP to have pseudo-orthorhombic symmetry; numerically, the

monoclinic angle differs from 90° by roughly $\frac{1}{2}^\circ$ only.

The result is that parts of a crystal may be pushed, literally or mechanically, from one monoclinic form into the other lying the other side of the orthorhombic ideal. The result is a twinned crystal. The patterns that Meeks and Auld have produced at the Applied Physics Department at Stanford University are nothing but those



of crystals in which twinned structures alternate with each other across the width of the whole specimen.

All this is accounted for by the atomic structure of the crystals. NPP is $\text{NdP}_5\text{O}_{14}$, and the monoclinic forms of the material are built, on an atomic scale, from tetrahedra of PO_4 ions linked together by the commonality of oxygen atoms.

The basic building-block of the crystal structure is a more or less flat ring of eight tetrahedra; these units are linked to other identical rings by means of pairs of phosphate tetrahedra. The result is a ribbon of linked phosphate tetrahedra some $13\text{ }\text{\AA}$ across and arbitrarily long. In the bulk crystals, which are cemented together by the appropriate disposition of Nd ions, the ribbons are laid down parallel to each other to form sheets, and then these sheets are piled on top of one another.

The origin of the ferroelasticity of NPP lies in the asymmetry of the eight-membered phosphate rings, which are so constructed as to bring one pair of oxygen atoms (on opposing tetrahedra) too close together for comfort. The result is a distortion of the ring from the most symmetrical arrangement that might be attained. The distortion is only small and, as might be expected, it disappears as the temperature is increased, which is why monoclinic NPP is transformed into rhombic NPP at a little above 140°C .

The formation of twins in such a crystal is then easily accounted for. The distortion of the basic phosphate ring in the crystal structure can be accomplished either by moving the tip of one of a pair of opposing tetrahedra to the right and the

other to the left, or vice versa. So one part of a monoclinic crystal may consist of rings distorted in one sense and the remaining rings distorted in the other. The horizontal stripes in the figure are such twins.

Because of the layered structure of NPP, however, it is clear that another form of twinning is possible, essentially because the registration of successive planes of phosphate ribbons can be changed relative to each other. Twinning of this kind, which is not in practice as easily accomplished, accounts for the vertical boundaries in the illustration.

The trick that Meeks and Auld now boast of is that for tuning the periodicity of the wedge-shaped alternating domains of crystal structure in twinned relationship to each other. Generating a pattern (such as that in the illustration) is largely a matter of supporting a plate-like crystal at two points and pressing between the supports.

The use of the word domain is deliberate; Meeks and Auld point out that the effects of a first application of stress are to cause the formation of a number of flat lens-shaped bubbles of different twinned crystal structure which, like ferromagnetic domains, appear to repel each other, so that their spacing is reasonably regular. The greater the shear stress on the crystal, the smaller the spacing. When the bubbles fill the space between the pressure points, they coalesce into a zig-zag pattern as in the figure, and when the stresses are removed, that part of the pattern outside the pre-existing stress pattern will be found to be permanently preserved between two immobile vertical twinning walls.

Tuning seems to be similarly straightforward. To increase the spatial frequency of a pattern of stripes, it is merely necessary to regenerate the original stress pattern and to move whichever stress point lies near the vertical boundary so as to compress the area occupied by the zig-zag pattern.

As things stand, NPP crystals are plainly a long way from being practical devices with any kind of function. The regularity of the spacing of alternating wedges of twinned crystal has, however, been confirmed by the demonstration that they are capable of yielding sharp Bragg diffraction spots (with optical light, not X-rays). The interest of a laser which is also a tunable optical grating will require time to be fully appreciated. Intrinsic unprecedented collimation is presumably only one of the potential benefits. **John Maddox**

Ecology

Water temperature and fleas correlated in Lake Windermere

from John H. Steele

WE ARE becoming much more conscious of, and interested in, changes in climate — both the long-term trends and the shorter fluctuations. Although the most direct evidence derives from records of temperature, wind or rainfall, it is often necessary to use biological indicators, such as tree rings or changes in composition of aquatic communities. But that raises questions of the relation between physical and biological variability, witness the arguments about whether 'punctuated equilibrium' is internally driven by ecological or evolutionary processes or is caused by external or even extraterrestrial physical events. Thus direct evidence of relations between climate change and biological patterns, such as the correlation reported on page 536 of this issue between the water temperature in June of Lake Windermere, in northern England, and the abundance of the dominant zooplankton, *Daphnia hyalina*, is always welcome.

Both the temperature and plankton data presented by D.P. George and G.R. Harris show a ten-year cycle over the period 1940–80. There are two other interesting factors noted in the paper. First, the population of the dominant fish, the perch, does not have a similar pattern, indicating to the authors that the fish neither influence nor are influenced by plankton density, at least on these time scales. Second, the ten-year cycle is similar to that observed in the northeast Atlantic, implying a large-scale relevance to the lake's physical and biological data.

Variability in biological data can be related to internal features of the ecological system as well as to external forcing. There has been a longstanding interest in using simple ecological models to investigate cyclical behaviour in populations but there is little acceptable evidence of how this actually happens without changes in the populations' environment. There are still arguments about the explanation of the lynx-hare cycles in the Arctic and the fairly regular outbursts of the spruce budworm. Cyclical behaviour in a particular time series of data may also disappear or be less obvious when more data become available. Thus an ecological system may appear to 'resonate' with its environmental forcing before lapsing back into irregular variations. A ten-year cycle in a forty-year data set raises questions that may be answered only by more observations or by specific causal explanation.

Equally intriguing is the authors' comparison of the temperature cycle in Windermere with that in the eastern North Atlantic, so that the lake data "reflect a

much wider pattern of climatic variation" with a period of approximately ten years. Though one's first thought may be of a possible relation to sun-spot cycles, air temperature records for central England over the past 300 years do not show this periodicity (Shapiro, R. *Q. J. R. met. Soc.* **101**, 697; 1975). Furthermore, a record of sea temperatures off Plymouth over ninety years shows quasi-cyclical changes that go in and out of phase with the sun-spot cycle (Southward, A.J. *Nature* **285**, 361; 1980).

If the correspondence in lake and sea temperatures is not mediated directly by air temperature, what other factors might be involved? In an analysis of the changes between 1873 and 1972 of the intensity of westerly winds over the North Atlantic, T.J. Makrogiannis *et al.* (*J. Clim.* **2**, 159; 1982) found, although they could not

easily explain, a ten-year periodicity. As George and Harris suggest, the link between temperatures in Windermere and the open ocean may arise indirectly through comparable variations in wind-induced mixing in the two very different aquatic systems. Again, there are questions about which processes in the ocean and atmosphere could generate such long-period cycles. Is there a northern oscillation like the southern oscillation associated with El Niño? It must be noted that all the longer-term data sets have several spectral peaks at frequencies from months to a few decades. Thus the interactions between oceans, atmosphere and lakes occur over a wide temporal range and will affect the biology in different ways.

These correlations between physical and biological events and between the open ocean and a mountain lake are tantalizing. Raising more questions than they answer, they demonstrate the need not only for longer data sets in aquatic environments but especially for studies of the causal interactions between land, ocean and atmosphere. □

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Cosmology

How did the Universe begin?

from I.G. Moss

BY AND large, cosmologists have become accustomed to the idea that the Universe began with an initial singularity of infinite density and infinitesimally small volume. This idea is strongly supported by the singularity theorems which Penrose and Hawking produced in the 1960s. But now, in an about-face that has come from a consideration of quantum mechanical effects on the origin of the Universe, Hawking and colleagues suggest that the Universe had a non-singular beginning followed by a period of very rapid expansion (which goes under the name of inflation) and then a transition to the conventional development described by the hot big-bang model. A longstanding problem of cosmology, to explain the origin of the density fluctuations that are needed to account for galaxies, is solved along the way by quantum fluctuations².

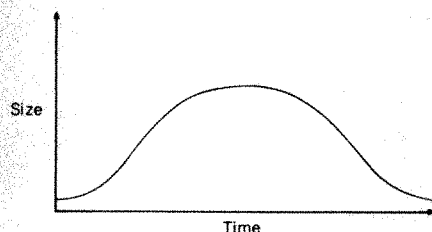
The problems of combining quantum mechanics with Einstein's theory of gravity still remain to be solved, but there does exist a coordinate-independent formalism of quantum mechanics (that is, one consistent with general covariance) invented by John Wheeler³ and Bryce DeWitt⁴. This very beautiful theory deserves to be better known. The wave function is a function of the geometry of space and of the matter fields. As a consequence of general covariance, the wave function satisfies a wave equation, known as the Wheeler–DeWitt

equation, which turns out to be the gravitational analogue of Schrödinger's equation. Thus, choosing the boundary conditions for this differential equation is analogous to choosing the initial conditions in a classical treatment of cosmology.

It was hoped that there would be only one solution of the Wheeler–DeWitt equation consistent with reasonable regularity conditions on the wave function but, as yet, there is no evidence that this is the case. Instead, Stephen Hawking has formulated a principal of quantum cosmology which produces one solution with particularly nice properties⁵. This wave function is constructed from the sum of many amplitudes for space-times with finite volume but no boundary. Hawking describes this by the cryptic statement⁶: "The boundary condition of the universe is that it has no boundary". His wave function is non-singular at space-time singularities and also has the property that it puts the matter fields into their ground state initially.

The first calculations of the wave function for the inflationary universe using Hawking's principle^{7,8} were performed on a model of the universe in which only a very small number of degrees of freedom are included (mini-superspace). These wave functions show recognizable features that correspond to the inflationary era and the matter-dominated era of

the universe. According to semi-classical approximations, they seem to correspond to classical universes that start out from a fixed size, at first expand exponentially but thereafter expand in the cycloidal form of matter-dominated universes, as shown in the figure. The wave function can therefore be interpreted in terms of universes which nucleate from nothing and eventually return to nothing.



An interesting problem of the origin of the Universe concerns the nature of time. Because the Wheeler-DeWitt formalism is coordinate free, time can only enter implicitly by the identification of some degrees of freedom as a kind of clock. If the volume of the universe is used as a clock, then it can be shown by a suitable approximation that the Wheeler-DeWitt equation reduces to the familiar Schrödinger equation for the matter degrees of freedom. In this case, we recover the standard physical models. However, the approximation breaks down when the universe is very small and it seems that the concept of time as we know it also breaks down.

A remarkable property of the Hawking wave function is that it is time-symmetric in the sense that probabilities for matter-field configurations are independent of whether the universe is expanding or contracting. According to Hawking⁶ this implies that the arrow of time defined by entropy increase would be reversed during contraction, which is reminiscent of the ideas of Gold and others in the 1960s.

For a classical universe it can be shown that this implies a boundary condition at the moment of maximum expansion¹⁰, which is unusual because it lies in our future. This interpretation is not forced upon us if the contracting phase collapses to a point: the wave function may then still be time-symmetric because it includes contributions from many classical universes. Half of these are completely time-reversed, but seem normal to time-reversed observers living inside them.

There still remain problems with quantum gravity. Fortunately, since these come from the interactions between gravitons, a treatment of the graviton degrees of freedom in a linearized approximation is possible. This calculation has been performed by Hawking⁷ among others. The result is that the amplitudes of these wave-like degrees of freedom have a gaussian probability distribution. The variance of this distribution corresponds to a scale-free spectrum of density fluctuations, which is quite acceptable for an explanation of the large-scale structure of the

universe, including galaxies.

On length scales larger than 100 Mpc, these inhomogeneities are still small at the present epoch. Therefore Hawking's principle of quantum cosmology, taken together with inflation, implies that the Universe is homogenous when averaged over large length scales.

Another difficulty is the interpretation of the wave function of the whole Universe, which may be expected to include observers of the Universe such as ourselves. If this is so, it leads to a 'many-worlds' interpretation of quantum mechanics. Furthermore, it turns out that the Hawking wave function is a superposition of quantum states that are peaked around different classical solutions of the form shown in the figure. Our view of the Universe is therefore as much a consequence of the complicated interaction between ourselves and the rest of the Universe as it

is a consequence of the state of the whole Universe. When calculating the spectrum of density perturbations, for example, there is a possibility that fluctuations between different classical universes may affect the result. □

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Proto-oncogenes

Clues to the puzzle of purpose

from J. Michael Bishop

THE oncogenes of retroviruses arose by transduction of normal cellular genes that are in consequence known as proto-oncogenes. What are the physiological functions of proto-oncogenes, why have they been conserved across phylogenetic time, and why do their viral offspring wreak such havoc? The most conspicuous property of viral oncogenes is their ability to impose mitosis on cells that might otherwise be at rest. It seemed reasonable then to suppose that proto-oncogenes would also encode mitotic signals. But a story now emerging suggests that this is not the case for *c-src*, the first proto-oncogene to be discovered. Two papers in this issue provide the latest chapters^{1,2}.

Cotton and Brugge began the tale by showing that pp60^{c-src} (the protein-tyrosine kinase encoded by *c-src*) is particularly abundant in brain, retina and spinal ganglia — tissues not renowned for their mitotic activity³. Sorge *et al.* followed with the demonstration that differentiated and mitotically silent neurones are responsible for the abundance of pp60^{c-src} in the retina⁴. Whatever the function of *c-src* may be, it is apparently required in cells that are no longer dividing. Brugge and her colleagues have now extended this conclusion to include both neurones and astrocytes cultivated from the central nervous system of rats and, in the process, have added new complexity to the plot¹.

The pp60^{c-src} protein isolated from neurones displays two distinctive properties: a presently mysterious structural modification in its amino-terminal domain⁵; and substantially greater specific enzymatic activity than in other tissues¹. Changes of this sort are familiar to mavens of *src*. Phosphorylations of tyrosine in

the amino-terminal domains of pp60^{c-src} and pp60^{v-src} (the product of the viral oncogenes, *v-src*) seem to increase the kinase activity of the proteins^{6,7}; the kinase activity of pp60^{c-src} is enhanced by binding to the middle T antigen of polyoma virus^{8,9}, perhaps by an influence on post-translational modification⁹; and the basal enzymatic activity of pp60^{c-src} is reputed to be well below that of its viral cognate¹⁰. If there is a unifying theme here at all, it seems to be that post-translational modifications brook large in regulating the physiological activities of *c-src* and *v-src*. But simple changes in activity may not be the whole story, since *c-src* alone is unable to transform cells to neoplastic growth, no matter how robust its expression^{11,13}. The mutations that distinguish *v-src* from *c-src*¹⁴ apparently endow the viral gene with the ability to transform cells and cause tumours¹², and this could well be due to novel substrate specificities conferred on pp60^{v-src}.

Brugge and her colleagues believe (but cannot yet prove) that the structural modification of pp60^{c-src} they have discovered in neurones is responsible for its increased specific activity, though the modification is not phosphorylation of any sort. Their findings further dramatize the contrast between *c-src* and *v-src* by demonstrating again that perhaps no extreme of protein phosphorylation by pp60^{c-src} can elicit cellular proliferation; and they reveal a duality of control over *c-src*, since there is abundant expression of the gene in both neurones and astrocytes, but post-translational modification and augmented kinase activity of the gene product only in neurones. These subtleties exemplify the sorts of fine-tuning that operate to govern

the performance of cells.

Is *c-src* master or servant? Does it command any of the events essential for the differentiation of neuronal cells, or does it merely help to sustain the routine metabolism of the cells once they have reached a preordained point in development? Alemà *et al.* have addressed this issue tangentially by deploying *v-src* in PC12 rat pheochromocytoma cells, which assume at least some properties of sympathetic neurones when exposed to nerve growth factor². It is already known that *v-src* can meddle with differentiation in a variety of developmental lineages, either blocking or reversing differentiation¹⁵. Alemà *et al.* now complete the cycle (to use a metaphor that will reverberate fully only for cognoscenti of American baseball) with their discovery that expression of *v-src* in PC12 cells, on the contrary, induces differentiation without assistance from nerve growth factor. The phenotypes elicited by *v-src* and nerve growth factor appear quite similar, within the confines of a rather limited survey of markers. One point of interest that apparently has yet to be examined is whether the effects of *v-src* on PC12 cells are reversible. Does *v-src* drive the cells beyond a point of no return in the schedule for development, or are the pleiotropic (and potentially reversible) effects of phosphorylation by pp60^{src} directly responsible for the phenotypic changes that connote differentiation? The question could presumably be answered by the use of conditional mutants of *v-src*, much as Alemà *et al.* have used such mutants to prove that *v-src* is responsible for differentiation in the first place.

The potential parallels between the findings with PC12 cells and the expres-

sion of *c-src* in neurones are obvious. The modified form of pp60^{src} found in neurones could be a functional analogue of pp60^{src}, the effect of *v-src* on PC12 cells a facsimile of the role played by *c-src* in normal differentiation. There is an important caveat, however, that Alemà and his colleagues acknowledge but perhaps dismiss too lightly. The products of *c-src* and *v-src* are not physiological equivalents. The actions of *v-src* on cells are those of a mutant gene, demonstrably anomalous and thus ill-suited to exemplify the functions of *c-src*. We need the sorts of clues provided by the work reported here. But more decisive means will be required to solve the puzzle of purpose that has been posed since the moment *c-src* first came into view. □

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Tropical diseases

Progress on *Theileria* vaccine

from Peter C. Doherty and Ruth Nussenzweig

EAST COAST FEVER (ECF), caused by the blood-borne protozoan parasite *Theileria parva*, kills approximately 500,000 cattle each year in Eastern and Central Africa. The task of finding a solution to this problem by contemporary scientific methods has been given to the International Laboratory for Research on Animal Diseases (ILRAD), which started in Nairobi 10 years ago and currently employs about 40 scientists working in collaboration with other groups involved in research and field-control measures throughout the ECF-endemic area of Africa, and with scientists in Europe and the United States.

The approach to solving ECF is determined by the biology of *T. parva*. The parasite is transmitted by the three-host tick *Rhipicephalus appendiculatus*, which feeds on infected cattle and ingests erythrocytes containing piroplasms. Buf-

falo, which are much less susceptible to the disease than are cattle, act as a wild-life reservoir. The parasite undergoes various developmental stages in the tick, culminating in the development of sporozoites in the salivary gland. The sporozoites infect other cattle through tick bites and rapidly invade lymphocytes, which replicate in concert with parasite schizonts and essentially become leukaemic cells. Enormous numbers of these lymphoblasts are formed, followed by massive lympholysis, extreme debility and, in most cases, death of the host animal 2–4 weeks after infection. It is not known whether T and B cells are targets for transformation *in vivo*, but all transformed cells differentiate to express T-cell surface markers, including thymus-leukaemia-like antigens. Tumorigenicity is a function of the continued presence of the parasite, because treatment of cloned cells *in vitro*

with cytochalasin B or theileriocidal drugs results in loss of transformed phenotype in the progeny cells that no longer contain schizonts.

The first obvious place to interrupt the parasite cycle immunologically is to prime cattle to reject the tick, but this approach has had only limited success. Recent efforts have been made to immunize cattle against the sporozoites. Mapping the schizont with a battery of monoclonal antibodies indicates that there are at least four different strains of *T. parva*, but monoclonal antibodies against the sporozoites do not distinguish between the strains^{1,2}. Furthermore, these antibodies, and sera from immune cattle³, will neutralize sporozoite infectivity *in vitro*. Such neutralization also seems to occur *in vivo* in cattle with high titres of anti-sporozoite antibody, although more satisfactory experiments to address this point are in progress. The target for the relevant antibody is evidently a molecule of relative molecular mass 68,000 which seems to be expressed both internally and on the surface of the sporozoite. Ultrastructural analysis has shown that the parasite surface antigen transfers to the plasma membrane of the host cell as the sporozoite invades, and remains on the surface of the lymphocyte for some time. Biotechnology techniques will allow large amounts of the sporozoite molecule to be produced for further analysis and possible vaccine production; both complementary DNAs and genomic libraries have been prepared and experiments are in progress to identify the relevant genes.

The obvious problem for the development of a sporozoite vaccine is to induce a level of immunity such that insufficient sporozoites escape neutralization and so establish a lethal infection. Cattle can, however, control infections induced experimentally by small numbers of autologous schizont-infected cells⁴. The alternative is to concentrate on eliminating the schizont-transformed lymphocyte. The infected cells can be killed by *Theileria*-specific, major histocompatibility complex (MHC) restricted, cytotoxic T lymphocytes (CTL)^{5,6}. Such effectors are readily generated *in vivo*, although there are problems in maintaining CTL specificity following restimulation of primed lymphocytes *in vitro*. Part of the difficulty for ILRAD is that it must develop the basic reagents and techniques for prosecuting bovine cellular immunology. In this context, ILRAD hopes for some technology transfer from Africa to the developed countries.

As with many murine CTL systems, there has been no success in developing monoclonal antibodies that detect *Theileria* parasite antigens on the surface of transformed cells. Identification of the relevant gene product may only be achieved by the application of molecular biology, as was also the case for some murine systems^{7,8}.

A genomic library has been constructed from piroplasm-infected red blood cells in a λ -phage expression system and is being probed with monoclonal antibodies directed at schizont antigens. If the system is similar to that of influenza virus⁹, the CTL may be specific for changes induced by a parasite molecule that is readily detected within the cell by antibody binding but is not easily demonstrated on the outside of the plasma membrane.

Assessment of the relevance of any schizont gene product for T-cell recognition required the solution of several technical problems. The first is the supply of readily available CTL clones for assessing antigenicity. The techniques for establishing concanavalin A-stimulated and alloreactive T-cell clones from cattle have now been established at ILRAD, so this is unlikely to prove a long-term difficulty. The second is the need for class I MHC-compatible bovine cells that can be transfected with parasite genes and then tested with such CTL clones. An alternative may be to exploit the vaccinia virus gene transfer system which has worked well with, for instance, human hepatitis¹⁰. Virus vector technology may ultimately provide the solution to the delivery of both sporozoite and schizont genes to exposed cattle.

Genetic engineering approaches to developing both sporozoite and schizont-specific vaccines offer the possibility of a long-term solution which could take 5–10 years to perfect. A more immediate control measure is already available; that is, to infect cattle with sporozoites and treat them concurrently with long-acting oxytetracycline, providing a solid immunity (at least to homologous strains). The availability of schizont-specific monoclonal antibodies for analysing cross-protection patterns and for typing strains endemic to a particular area, together with the development of better drugs for treating the early stages of ECF, make this a feasible protocol.

There are good reasons for using the

infection-treatment method only as an interim procedure. One is that the sporozoites for infection can only be supplied as an homogenate of tick salivary glands. There is also currently no satisfactory way of titrating *in vitro* for vaccine standardization, although there are possibilities of using anti-sporozoite monoclonal antibodies or a lymphocyte binding assay. Another difficulty is that delivery of such a vaccine in many field situations is both cumbersome and demanding. Even so, the procedure could be economically feasible as there would be less need for costly dipping to control ticks. Unfortunately early indications are that a reduction in dipping of ECF-immune cattle leads to the emergence of other tick-borne diseases. Two such diseases, babesiosis and anaplasmosis, are the subjects of investigation in other laboratories but the third, heartwater (caused by the rickettsia *Cowdria ruminantium*), is at present little studied.

Has the experiment of ILRAD been a success? It has made initial progress in the

analysis of ECF and is now a focus for research training and contemporary biotechnology transfer in Africa. It is also nurturing the emergence of well-trained African researchers, and giving a number of young scientists from the developed countries the opportunity of first-hand experience in working with disease problems of this nature. The answer, then, must be yes. □

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Archaeology

Early man as complex hunter

from Clive Gamble

PREHISTORIC archaeology is beginning to show that human societies dependent on foraging and hunting existed at different levels of organizational complexity. This conclusion is far removed from the generally accepted notion that all hunting and gathering societies were the same and did not change until the appearance of agriculture.

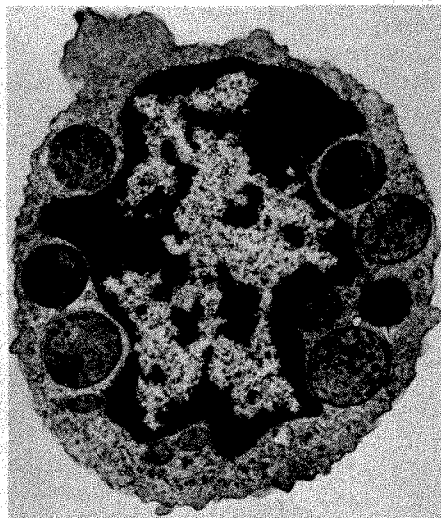
Although research into the origins of hominids and human culture has captured most attention, equally important, but less publicized, advances have been made in studying the archaeology associated with *Homo sapiens sapiens*. For most parts of the world this covers a period starting at least 40,000 years ago, except in North America, where R.D. Guthrie¹ argues that the earliest colonization occurred about 12,000–14,000 years ago.

The comparative study of hunter-gatherers on a world scale began over 20 years ago with the symposium *Man the Hunter*². Many of the concepts emerging since then have formed the basis for research that has dramatically changed archaeological practice and analysis. These include ethnoarchaeological studies of modern foraging groups, performed most productively in Alaska, the Kalahari and many parts of Australia. R. Lee (in ref. 2) used a world sample to examine the importance of particular subsistence activities — hunting, fishing and plant foraging. This work was the starting point for many studies of primary adaptive behaviour related to resource management and procurement. Such behaviour is now commonly discussed in terms of a

world model of hunters and gatherers, where variation in food-searching behaviour is related to survival strategies to cope with the differential distribution of energy and usable food resources in global environments. In archaeological terms, these highly differentiated strategies left behind distinctive signatures, as measured by the density and distribution of flint artifacts within regional territories, evidence that groups used the tactics of mobility, group size and composition and inter-group alliances as buffers to enable them to survive in the Pleistocene.

At this symposium, M. Sahlins (in ref. 2) characterized hunters and gatherers as the 'original affluent society', based on the short working week of some of the Kalahari and Arnhem Land societies. Affluence has also been recognized as a feature of coastal groups in north-west North America. Here, bulk storage of salmon apparently provided a subsistence base for the elaboration of material culture, a high degree of sedentary behaviour and the degree of social ranking (including slaves) in these societies. Such societies' lifestyles have frequently been regarded as exceptional in comparison with the nomadic style of other contemporary foraging groups.

Such societies have been re-examined recently³ in terms of the emergence of cultural complexity in the New and Old Worlds. Cultural complexity is measured through population density; widespread exchange systems using raw materials as well as items as diverse as pottery vessels, ear-spools and alligator teeth; evidence



Electron micrograph of a bovine lymphocyte invaded by several sporozoites of *Theileria*, $\times 10,000$ (courtesy of ILRAD).

for permanent and larger settlements; the appearance of artistic codes used to transfer information; and harvesting mechanisms such as eel traps, which require inter-group organization to use efficiently the high-energy, small-package food supplies in the low trophic levels. Some of the clearest measures of complexity come from the analysis of cemeteries like that at Oleneostrovski mogilnik in northern Russia, where the differential distribution of grave goods and the spatial location of individuals indicate hereditary social positions among this group of Holocene boreal forest foragers⁴.

These findings confirm L. Binford's⁵ earlier characterization of the stone tool industries of the European upper Palaeolithic as reflecting a relatively complex social geography compared with earlier traditions. M. Conkey argues that it is no longer possible to characterize societies of the Upper Palaeolithic or Mesolithic periods by a single social type (in ref. 3). Instead, there existed a mosaic of social structures bound together on a large spatial scale by ramifying networks of alliances. Consequently, complexity has to be measured at both a regional and continental scale. As B. Bender (in ref. 3) points out, the characteristic complexity in these worlds of hunters and gatherers was for shifting emphasis within a regional landscape, as power structures emerged at a local level for relatively short periods of time. These must be compared with the world that hunters and gatherers are found in today and in the historic and later prehistoric pasts. Here, any transformation to complexity has occurred within the arena of a much more complex social geography, including state organizations of immense elaboration and complexity⁶.

Recent work has thus swept away lingering notions that complexity and intensification in social and economic behaviour arrived with the introduction of agriculture. The archaeological definition of agriculture is now unclear, as prehistoric hunting and gathering societies expanded to fill the ethnographic spectrum that has previously been accorded to their more 'sophisticated' successors.

The investigation of the causes for the emergence of cultural complexity is still beginning. One fruitful line of enquiry, proposed by G. Johnson⁷, is that of scalar

stress, which arises as groups settle down and form larger integrated units. This results in information-processing problems when more than six such units have to be integrated. One solution is the inauguration of hierarchies, as they are more efficient at processing information and hence solve some of the problems associated with sedentism.

D. Brown and J. Price conclude³ that what emerges from these discussions of cultural complexity will define the archaeological units of analysis on which the locus of explanation will eventually settle. There are two such units: the first emphasizes the social aggregate and is investigated at the macrosystemic level, which can be done by investigating sites in regions and interpreting them as group residues; the second is the social 'molecule', where selection occurs at the level of the individual and family and minimizes group stress in the short term. This microsystemic approach still awaits the development of archaeological methodology to realize its full potential. The combination of both scales may elucidate changes occurring in the varied timescales of the archaeological record.

When viewing complex societies from

the perspective of the hunter-gatherer, agricultural communities appear to be the failures, rather than the successes, of prehistory in that they had to invent or adopt agriculture, because their systems were breaking down both at the group and individual scale. Out of such stress came the complexity that is now recognized in the archaeological record. The challenge is to understand precisely how present-day models have previously prevented us from appreciating the diversity of the prehistoric world of the hunter and gatherer by obscuring the questions at the very heart of archaeological inquiry — why does change occur and why does variation in human societies exist? □

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Drilling technology

Well-loggers and scientists

from Roger N. Anderson

DRILLING for oil is fundamentally different from drilling for scientific purposes. Although an understanding of the geology penetrated by the drill bit is a requirement for the discovery of new oil and gas reserves, the economics of drilling have caused the oil industry to develop an extremely sophisticated remote method for determining the physical and chemical states of rock after they have drilled through it as fast and as deep as they can. It is simply too expensive to cut core samples during the drilling of an oil well. This process eliminates the traditional primary product required by geologists for scientific investigation — the core — and substitutes the 'log'. A recent symposium* examined the differences that are inherent in logging for purely scientific as opposed to commercial objectives.

The technologically advanced well-logging service is possibly the single most important operation in the oil industry, as well as being the most profitable. The devices monitor electrical, sonic and nuclear measurements made up and down an oil well from the surface, producing a log of the geology without the need for samples. These logs detect lithology, formation strike and dip, porosity, fractures and, most importantly, the chemical composition of the fluid filling the pore spaces of

the rock — that is, they can show whether oil and gas are present.

In contrast, drilling deep into the Earth for scientific purposes has historically depended on core for the determination of these geological characteristics. Without the pressure of time, or the requirement of great depth, scientists can slowly cut their core as the well progresses. Most scientific drilling has been the result of the Deep Sea Drilling Project, where holes are relatively shallow even though they are beneath great depths of water. Now that several countries are involved in deep continental scientific drilling, the high costs of coring are causing the scientific community to devote more attention to how logs can be used to supplement and support coring for the determination of the geology of the deep crust. Interestingly, logs measure parameters at different scales than do cores and the two fit together to increase greatly the amount of data returned per foot of well drilled.

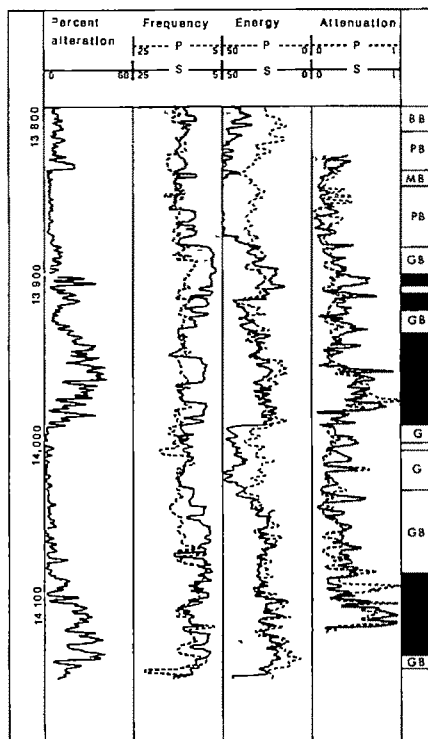
Often, an oil company's 'noise' is a scientist's 'signal'. Take the most sophisticated of well logs developed to date — nuclear bombardment of the rock. New techniques presented at the symposium by Gordon Pirie, Mike Herron and Charles Flaus (Schlumberger) showed that true neutron activation analysis of the elemental chemistry of the rock can now be accomplished with a logging tool. At



Feast among the Kwakiutl Indians of the north-west coast of North America. A feature of the feast was the destruction of goods, which led to gain in status and prestige (courtesy of the Smithsonian Institution).

*'New Techniques in Scientific Well Logging', Lamont-Doherty Geological Observatory, New York, 15-16 May 1985.

Example of the new types of scientific log being developed. The nuclear and sonic logs for the depth interval 13,800–14,150 ft (below sea level) are shown for Deep Sea Drilling Project hole 556, which cuts through 37-Myr-old oceanic basaltic crust on the western flank of the mid-Atlantic ridge. The column on the left shows the degree of hydrous alteration of the basalt (strictly a measure of the chemically bound water in the rock). This is derived from determinations of the radiation arising from bombardment of the rock walls with gamma rays and neutrons from sources put down the hole. The other three columns show, from left to right, the centre frequency (in kHz), energy (in dB) and energy attenuation (in nepers per foot [1 neper = 0.8 dB]) of the refracted P (compressional) and S (shear) wave signal from a 10 kHz broadband source. The solid and broken lines are the P- and S-wave logs respectively. On the right, the lithological section from the core rock. BB, basalt breccia; PB, pillow basalt; MB, massive basalt; GB, gabbroic basalt; G, gabbro; black, serpentinite. Two serpentinite dykes show up clearly from their high alteration content and increased attenuation. The combined use of nuclear and sonic logs enables the chemical and rheological properties of the rocks to be determined without taking a core.



present, 11 elements can be 'counted' with a scintillation crystal, and this will soon increase to 30 or more with the development of cryogenic germanium counters. The oil industry, of course, concentrates on the determination of carbon and oxygen ratios in the pore fluid, whereas the geological community will use this tool for direct rock chemical measurements.

Nuclear magnetic resonance imaging similar to that being developed for medical use was described by Chuck Newman (Chevron). Here, a large magnetic field polarizes the magnetic poles within the pre-fluid and sensitive detectors measure the rate of precession of these dipoles from the oriented state back to their natural random state. This measurement is directly proportional to the permeability, or ability to flow, of the pore fluid. Scientific interest in this measurement is not in the ability of the fluid to flow into an oil well, but in the description of fluid movements within a convecting system, such as a hydrothermal circulation system within a volcano or a geothermal hot spring.

Other new logging technology involves imaging the wall of the drill hole by ultrasound. In a way that is reminiscent of the use of ultrasound to obtain non-invasive images of a fetus in the womb, ultrasonic sound is bounced off the well bore and presented as a 'television' image. Because wells are drilled with opaque mud, light cameras cannot be used. However, even if they could, they would not produce quantitative images comparable with ultrasound. Because the travel time of sound is so much slower than light, the waveform of the reflected energy can be digitized and its reflection amplitude and travel time displayed separately. Lithology, fracture locations, dip and bedding planes can be seen in the reflected image, but far

more information can be derived by the travel-time display. As Mark Zoback (Stanford University) showed, the state-of-stress within the Earth can be determined by this seemingly innocuous measurement. A display of travel time from source-firing to receipt of the reflected pulse simply gives a 360-degree picture of the size of the well bore. This caliper measurement reveals great forces pushing on the crust around the well bore from far away mid-ocean ridges, seafloor subduction zones and mantle convection. Zoback finds that borehole deformation is caused by the same forces that push and

pull the plates of the Earth's surface across each other. By sending ultrasonic sound pulses into the well, it is feasible to determine the forces which drive plate tectonics.

Seismic exploration from the surface had long been the dominant method used in the oil industry for the discovery of new subsurface accumulations of hydrocarbons. Ken Landgren and Roy Dove (Schlumberger), Joe Zemanek (Mobil) and T. K. Kan (ARCO) showed how these surface techniques can be extended into the drill hole to produce tomographic images of the geological structure surrounding the well, and to measure fundamental elastic properties of the rock such as shear-wave velocity and seismic attenuation *in situ*. Dan Moos (Lamont) and Dick Plumb (Schlumberger) demonstrated how images of the fracture distribution along the borehole can be related to sonic and seismic wave propagation along the borehole walls enabling fracture permeability of the formation to be determined.

Technology developed for a slightly different application can therefore be used in scientific exploration of the inner depths of the Earth's crust. Insight into the geology of the planet extending far from the approximately 12-inch hole cut into the rock is bound to be the result. Seldom are we presented with such an opportunity to divert already developed high technology to scientific purposes. Usually, science leads to new technology — here, technology promises to lead to new science. □

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Endocrinology

Has the prolactin inhibiting peptide at last been found?

from George Fink

REPRODUCTION in mammals depends on the secretion of the gonadotropins, luteinizing hormone (LH) and follicle stimulating hormone (FSH), and of prolactin at appropriate times and in appropriate modes and amounts. The secretion of these hormones from the anterior pituitary is controlled by the central nervous system and by negative and positive feedback actions of the gonadal hormones, the oestrogens, androgens, progesterone and inhibin. The central nervous control of the gonadotropins and prolactin is effected not by a direct neural link between the brain and the anterior pituitary gland but rather by neurohormones, released in the median eminence at the base of the brain, which are conveyed to the anterior pituitary gland in the exquisite hypophyseal

portal vessels. So far, the evidence is that gonadotropin secretion is stimulated by only one neurohormone, gonadotropin releasing hormone (GnRH). The neural control of prolactin secretion is much more complex and involves both prolactin inhibiting and releasing factors¹. Peter Seeburg and his associates now show that the carboxy-terminus of human placental GnRH precursor (Fig. 1) — termed GAP for GnRH-associated peptide — inhibits prolactin and stimulates gonadotropin secretion in primary cultures of anterior pituitary cells². Seeburg *et al.* suggest that GAP may be the sought-after prolactin inhibiting factor and argue that their findings simplify our understanding of the neural control of gonadotropin and prolactin secretion. But do they?

The most important function of FSH is to stimulate the growth and maturation of ovarian follicles in the female and spermatogenesis in the male. LH causes ovulation and the formation of the corpus luteum in the female and stimulates androgen secretion by the Leydig cells in the male. The ovulatory surge of LH is massive³ and can be triggered either reflexly by copulation as in the cat, rabbit, vole and tree shrew, or spontaneously, as in man, sheep and rat, by a positive feedback cascade (Fig. 2). Prolactin is primarily concerned with the production of milk and is therefore essential in mammals for the survival of the young after birth. In some eutheria, such as the rat, prolactin also maintains the corpus luteum (hence its older name, 'luteotrophin'), and in marsupials it virtually runs the whole reproductive cycle⁴. The 'basal' secretion of the gonadotropins and prolactin is pulsatile. In the case of the gonadotropins this is thought to reflect the importance of frequency modulation of gonadal function by the brain-pituitary system, seen most convincingly in seasonal breeders⁵.

Under certain conditions there is an inverse relationship between prolactin and gonadotropin secretion. In the human female, this is most prominent during lactation ('lactational amenorrhoea'), when plasma prolactin concentrations are increased several fold by a neurohumoral reflex triggered by suckling, and in patients who have pituitary tumours that secrete large amounts of prolactin⁶. The prolactin inhibitory action of GAP, Seeburg and associates argue², could provide a simple explanation for the inverse relationship between prolactin and gonadotropin secretion. Immunohistochemical evidence suggests that GnRH and its associated peptide coexist in hypothalamic neurones⁷ and the co-release of GnRH and its associated peptide would stimulate gonadotropin and inhibit prolactin secretion. Conversely, reduced or absent GnRH and GAP release into the hypophyseal portal vessels would lead to high prolactin and low gonadotropin concentrations in plasma.

The genetic engineering used by Seeburg's group² to synthesize GAP is beautiful in design and efficiency. Their model for the action of GnRH and GAP, although it has the attraction of simplicity, does not, however, explain or take account of several important facts. First, Seeburg's group use the rat, in which a

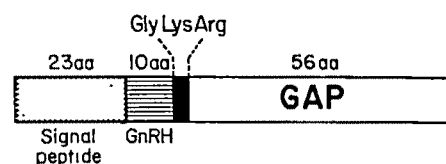


Fig. 1 The human placental precursor for gonadotropin-releasing hormone (GnRH) showing the 56 amino acid (aa) GnRH-associated peptide (GAP) separated from GnRH by the Lys-Arg dibasic cleavage site and the Gly amide donor (from ref. 2).

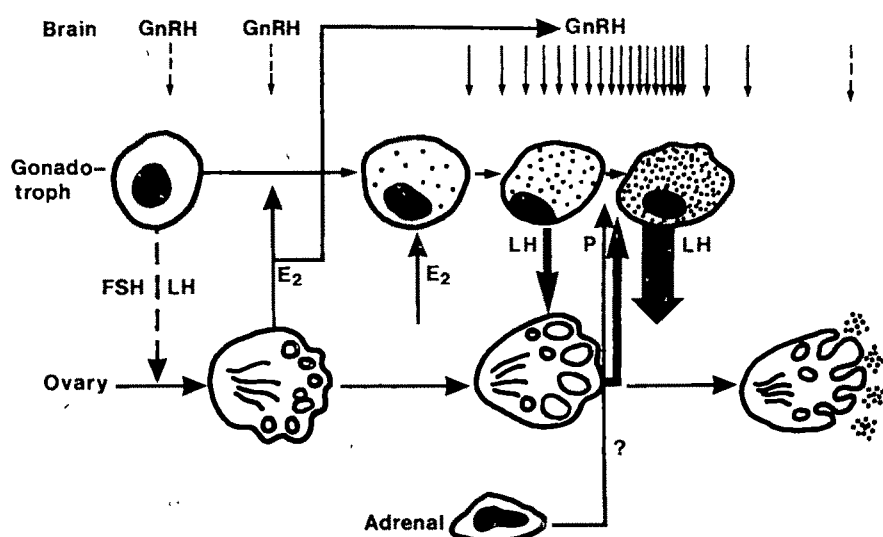


Fig. 2 Schematic diagram showing the positive feedback cascade which leads to the spontaneous ovulatory surge of LH, resulting in ovulation. 17 β -Oestradiol (E₂), secreted by the ovary under the influence of basal levels of gonadotropin, triggers a surge of GnRH from the brain by an action that involves monoaminergic and opioid mechanisms. It also stimulates an increase in the responsiveness of the pituitary gonadotrophs (increased stippling) to GnRH. Pituitary responsiveness is further increased by progesterone (P). The peaks of the surge of GnRH and the increase in responsiveness coincide because of the priming effect of GnRH which thereby ensures a massive surge of LH (from ref. 3 with permission of the British Council).

massive surge of prolactin occurs concurrently with the spontaneous ovulatory surge of LH, as well with the LH surge induced by steroids in several experimental models^{8,9}. But in women there is no evidence of a significant fall in the plasma concentration of prolactin at the time of the ovulatory surge of LH. Second, it is not at all clear whether lactational amenorrhoea is produced by volleys of impulses in the afferent (neural) limb of the milk-ejection reflex generated by suckling at the nipple, by the high plasma prolactin concentrations induced by suckling, or by both. Third, elevations in plasma prolactin concentration produced by pituitary grafts under the kidney capsule significantly reduce the plasma concentration of LH and FSH, probably by reducing both GnRH release into hypophyseal portal blood and pituitary responsiveness to GnRH. For, as the authors are aware, neuropeptides such as thyrotropin-releasing factor, vasoactive intestinal peptide and the closely related intestinal peptide porcine PHI are potent prolactin-releasing factors; immunoneutralization studies^{10,11} have shown that these peptides may play an important role in the neural control of prolactin release under certain physiological conditions.

Both the prolactin-inhibitory action and the gonadotropin-releasing properties of GAP must be carefully evaluated in *in vivo* preparations under physiological conditions before firm conclusions can be drawn. In Seeburg's primary pituitary cell cultures, GAP was about 10 times less potent than GnRH in stimulating LH release. The concentration of GnRH in hypophyseal portal blood at the peak of the ovulatory surge³ is about 10⁻¹⁰ M and so if it is to play a significant role in LH release much more GAP than GnRH

would have to be released from the same neurones. Perhaps GAP is more important for releasing FSH, for which it has a similar potency to GnRH, than LH; the differential release of GnRH and GAP could then explain in part the dissociation that occurs between LH and FSH release under certain conditions.

Notwithstanding the reservations about Seeburg's oversimplified model for the relationship between LH and prolactin release, the finding that GAP may be the prolactin-inhibiting factor is important and exciting. Until now dopamine has been considered to fill that role, especially in humans where administration of dopamine receptor antagonists such as chlorpromazine and other neuroleptics, marked increases plasma prolactin concentrations, whereas dopamine agonists, such as the ergot derivative 2-bromo- α -ergocriptine (bromocriptine), significantly reduce plasma prolactin concentrations. Bromocriptine has been used as an effective treatment for amenorrhoea and infertility produced by abnormally high plasma concentrations of prolactin. It remains to be seen whether GAP, or shorter and more potent analogues of GAP, will prove to be more effective □

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Labelling the axes of graphs . . .

The subject of how to express numbers on the axes of graphs — most particularly powers of ten — was the subject of letters from C. Liébecq (Nature 314, 586; 1985) and P. Luykx (Nature 315, 462; 1985). The discussion continues . . .

SIR—Liébecq and Luykx have identified a source of confusion and occasional error in the numerical tabulation of quantities. The author who wants to express concisely a quantity such as 1.5 million joules in a table has at least three choices for the column heading. They are: Energy $\times 10^{-6}$ (J); Energy (J $\times 10^6$) or Energy (MJ). In each case the numerical entry is 1.5. I suggest that the first two possibilities are correct but confusing. The third is to be preferred. A fourth possibility is to use the pure number created by dividing the quantity by its unit (Energy/MJ). Although internationally recommended, this option has been shunned by authors and editors alike.

Finally, may I express the hope that both your contributors were aware of the dual enormity of expressing "radioactivity" in "c.p.m.". Radioactivity is a phenomenon. The quantity is activity and its unit is the reciprocal second, named for this purpose the becquerel. Colloquially the unit may become transition/second, although the entity, transition (not count!), is implicit in the definition of the quantity and should not be duplicated in the unit — compare frequency in s^{-1} , no longer in cycles/second.

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SIR—In recent weeks this column has seen a complaint by Liébecq that a graph axis annotated with figures ranging from 1 to 10 and labelled "Radioactivity (c.p.m. $\times 10^3$)" is incorrectly labelled when one of the implied values is, for example, 5,000 c.p.m.; and a response by Luykx, arguing that the original labelling can be defended as long as one abandons an algebraic interpretation of the symbols. Luykx comments that "there ought to be agreement" on how to read the axis labelling.

It is clear that agreement has not yet been reached, but at least a completely rational system is available, and is particularly well described in the invaluable document *Quantities, Units and Symbols*, prepared by the Symbols Committee of the Royal Society and representing the recommendations of several international bodies concerned with the proper use of scientific notation.

The example above required the observation that if "Radioactivity = 5×10^3 c.p.m." then " $5 = \text{Radioactivity}/(\text{c.p.m.} \times 10^3)$ ". Thus, the right-hand sides of any of these last three equations are acceptable labels for an axis which includes the numerical value 5, whereas the expressions recommended by both

Liébecq and Luykx are not. Given the evident attraction of such an unambiguous system for labelling graph axes and table columns, it is indeed hard to understand why so many scientists (and all too many of the journals which publish their work) insist on using labelling systems which call for inspired guesswork on the part of the reader.

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SIR—Luykx concedes that Liébecq would be correct if ordinate labels were thought of as equations in the form "radioactivity = $5,000 \times \text{c.p.m.}$ ". Luykx also wishes that agreement could be reached about how such labels should be read.

On the first point, the opening, defining, sentence of the Royal Society's report *Symbols, Signs and Abbreviations recommended for British Scientific Institutions* (1969) contains the equation "physical quantity = numerical value $\times$ unit", that is, exactly in the form that Luykx considers illogical. On the second point, the Royal Society's document represents not only itself but also several leading British scientific societies including the (now) Royal Society of Chemistry and the Institute of Physics, and agrees with all of the major international bodies concerned, including IUPAC, IUPAP, ISO and CGPM. So it is clear that a considerable degree of consensus has existed for many years.

The confusion can be removed by always attaching the power to the physical quantity rather than to the unit; that is, $10^{-3} \times \text{radioactivity (c.p.m.)} = 5$ means that the radioactivity is 5,000 c.p.m. This journal (the *Australian Journal of Plant Physiology*) follows that convention as do many others. Of course, the problem can often be avoided by use of SI units and their standard prefixes.

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SIR—There have been two letters on the expression of large or small numbers on figure coordinates, the most recent by Luykx. He points out that there ought to be agreement on how to read them.

The *Journal of Bacteriology*, as with other American Society for Microbiology publications, follows the convention of expressing a large number such as, for example, 10^9 cells/ml, as 10 on the ordinate scale and 10^9 cells/ml or cells/ml (10^9) as the label. No " $\times$ " is used. This style is

readily understandable because it is comparable to the use of kg or mmol on a coordinate.

As to a standard, it is unfortunate that the Council of Biological Editors did not deal with this question explicitly. However, in the *CBE Style Manual* (Fifth edition, p. 79) they do set a style for units in tables, and it is this that has been adopted by the ASM for both graphs and tables.

In dealing with graphs, I suspect that the council could not agree among themselves. In the section of the manual dealing with illustrative material, 'Graphs' and 'Tables' are adjacent, and the latter includes this phrase, which in similar words can be traced back through earlier editions: "Except in rare instances, avoid numbers ($\times 10^6$), ($\times 10^{-6}$) as multiplying factors in column headings; they have caused much confusion in biological literature . . .".

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SIR—Luykx maintains that "quantity (unit $\times$ number)" is not incorrect. His defence of this form is based on viewing the bracketed expression simply as a descriptive label which he then interprets by identifying the unit-symbol with the numerical value of the quantity.

The point at issue is, however, not correctness versus incorrectness but rather clarity versus ambiguity. I and most of my colleagues use "quantity/(number $\times$ unit)" or "quantity/(prefixed-unit)" wherein the number or prefix may be a power of 10. This accords with the Royal Society, (*Quantities, Units and Symbols*, 1971), with the policy of the Conférence Générale des Poids et Mesures, and with the International Organization for Standardization with which, in turn, almost all national standardizing bodies cooperate. I find the carefully considered conclusions of these eminent bodies less ambiguous and generally more likeable than Luykx's arbitrary preferences. I observe that some "quantities" might cause me to replace our bracketed expression by a description such as "on the Beaufort scale".

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SIR—Liébecq and Luykx have recently suggested how to label the axes of a graph. May I suggest another method?

To continue with the example introduced by Liébecq, if one wishes to show radioactivity data in the range of 5,000 c.p.m., surely one should label the axis, "Radioactivity/ 10^3 c.p.m.", with gradua-

tions from 0 to 10. For a point lying halfway along this scale, the measured radioactivity divided by 10^3 c.p.m. gives 5, so the datum is clearly 5×10^3 c.p.m.

The approaches favoured by Liébecq and Luykx appear to have a common drawback. If on a graph or in a table one uses the heading, "velocity $\times 10^2$ (ms^{-1}), there is always the risk of some confusion. Should a reading on the graph or in the table of "48.5" be read as a velocity of $48.5 \times 10^2 \text{ ms}^{-1}$, or as $48.5 \times 10^{-2} \text{ ms}^{-1}$? Mathematical logic tells us it is the latter, but many people seem to get confused, some authors included. The use of the solidus, as exemplified above, has the advantage that the factor one needs to insert is the same factor as appears on the axis or at the head of a column, that is, "velocity/ 10^{-2} ms^{-1} ". Being completely unambiguous, this method is consequently less error-prone.

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More help required on T and B cells

SIR—I read with great interest a recent letter¹ and a *News and Views* article², concerning immunological help. It was reported that a B lymphocyte can pinocytose free toxin that it binds to and present digested fragments of it on its surface. It was also reported that a helper T lymphocyte which binds to these digested fragments can help the presenting B lymphocyte to differentiate to plasma cell form in which it secretes large quantities of antibody. In the *News and Views* article several unresolved questions relating to immunological help were raised. I thought of some questions which were conspicuously absent and will here raise them and suggest hypothetical answers.

First, how is a B lymphocyte helped if it binds to a surface antigen of a pathogen? B lymphocytes might find it difficult to pinocytose a surface of any pathogen unwilling to facilitate its own destruction, and B lymphocytes are not specialized to safely phagocytose a live pathogen. Digested fragments of a pathogen presented by macrophages would not be an adequate substitute since they would not include many of the original surface antigens. Perhaps when a B lymphocyte binds to an antigen that it is unable to pinocytose, it attracts a macrophage to come and phagocytose the attached pathogen or cell, and remains with the macrophage until it places digested fragments (of the antigen and the rest of the pathogen or cell) on its surface and the B lymphocyte's surface.

Second, how is it assured that B lymphocytes will be helped if and only if they bind to nonself antigen, while still allowing pseudorandom generation of T lymphocyte antigen receptors? Perhaps hel-

per and suppressor T lymphocytes are clones of T lymphocytes which differentiated prenatally to helper or suppressor forms according to whether they did not or did bind to digested fragments of materials presented by macrophages then.

Third, how is it assured that B lymphocytes will not be helped if they bind to B or T lymphocyte antigen receptors? The diversity of helper T lymphocytes might be greatly restricted if there could be none that bound to the many different digested fragments of these receptors. Perhaps macrophages have a special ability to recognize B and T lymphocyte antigen receptors and do not present digested fragments of them; either prenatally or postnatally.

Fourth, how is it assured that B lymphocytes will not cause serious damage to self materials during an infection if they bind to an antigen common to self and pathogen? Perhaps since activated T lymphocytes would be concentrated near the pathogens, so also would their mediating chemicals, the B lymphocytes they helped, the antibodies of those B lymphocytes and the leukocytes which destroy antibody coated objects.

I wish researchers would devise experiments which clearly address the questions raised and possibly test some of the hypothetical answers suggested.

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Hormone receptor-effector complex evolution

SIR—I wish to propose a theory for the evolution of receptor-effector complexes where the effector is a protein or peptide hormone. Recently, there has been much interest in the evolutionary relationship between peptide hormone effectors and their receptors. Hales proposed that peptide effectors are produced by proteolytic cleavage of membrane proteins that are evolutionarily related to receptors¹. Several recent findings concerning the peptide hormone epidermal growth factor (EGF) are consistent with this hypothesis. EGF is cleaved from a large precursor protein (prepro-EGF) that may reside in the membrane and appears to share some sequence homology with the human low-density-lipoprotein (LDL) receptor^{2,3}.

In a recent paper⁴ Baldwin noted that

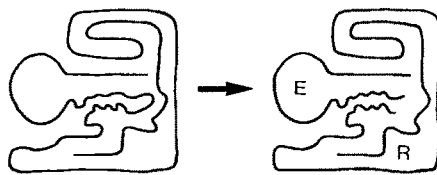


Fig. 1. A single polypeptide progenitor (left) becomes a two-polypeptide complex of receptor (R) and effector (E).

there was also sequence homology between the coding region in the oncogene *c-mos* and the precursor protein for EGF. This homology did not extend into the part of the polypeptide that was cleaved into the hormone. *c-mos* is functionally related to the avian oncogene *v-erb-B* and presumably evolved from the same gene^{5,6}. In turn, *v-erb-B* has substantial homology to the EGF receptor protein⁷. This means by virtue of the relationship of *c-mos* to *v-erb-B*, the precursor protein for EGF (prepro-EGF) would have to be distantly related to its receptor as well as contain the EGF peptide.

The structure of prepro-EGF leads me to extend Hales' ideas¹ and propose a model for the evolution of receptor-effector complexes. The model is based on the fact that receptors and their effector proteins form complexes due to protein-protein interactions. When a single polypeptide chain folds, different parts of the chain interact intimately with other parts of the same chain (Fig. 1). I propose that some of the genes encoding receptor-effector complexes began as single genes that encoded single polypeptide chains that were capable of intimate folding interactions. Receptors and their effectors then evolved due to changes in the DNA that split and sometimes duplicated the progenitor gene into two genes capable of encoding two distinct polypeptides, one the receptor and one the effector, that were still capable of interacting or folding with each other. For EGF and its receptor I envision that the progenitor gene was first duplicated. The copy that became the receptor deleted the domain encoding the hormone leaving a pocket for the missing domain encoded by the duplicated gene.

However, only gene-splitting, and not gene duplication, is obligatory of the general mechanism that I have proposed. After the gene is split, differential gene expression in different cell types could then induce the synthesis of each part of the original pro-polypeptide. The interaction of the two protein parts would lead to the formation of a complex capable of performing a biological task.

The interaction of these polypeptides could effectively lead to a cell-cell or hormone-cell interaction. Regardless of whether the relationship between prepro-EGF, *v-erb-B* and a *c-mos* is real or coincidental, the simplicity of this model bears further consideration as a general mechanism for the evolution of receptor-effector complexes.

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Geologists in conflict

Gordon L. Herries Davies

The Great Devonian Controversy: The Shaping of Scientific Knowledge among Gentlemanly Specialists.

By Martin J. S. Rudwick.

University of Chicago Press: 1985. Pp. 483. \$39.95. To be published in Britain in September, £36.75.

THIS is a definitive study — a work which is going to be accorded classical status. Its field, the history of the earth sciences, will never again be quite the same. So many previous historians within the earth sciences have chosen the expansive approach; they have painted in broad brush-strokes upon gigantic canvases. In 1905 Sir Archibald Geikie traced the history of geology from the speculations of Aristotle to the petrography of Henry Clifton Sorby in a mere 473 pages; in 1982 Professor Mott Greene told the story of geology from the Huttonian theory to plate tectonic theory within a volume of similar compass. Such works of sweeping purview clearly have their place, but hitherto our literature has contained few detailed micro-studies of those really crucial episodes which have punctuated the history of the earth sciences and endowed them with their present character.

Rudwick, with one major work already behind him (*The Meaning of Fossils*, first edition 1972), has sought to remedy this lacuna by focusing narrowly upon one important but now largely forgotten episode. That episode is the so-called Devonian Controversy which engaged the attention of some of the world's leading geologists during the years between 1834 and 1843. To the events of those eight years he devotes almost 500 pages and his microscopic analysis reveals exactly how there emerged the concept so familiar to us as the Devonian System. He has been able to accomplish his task because in addition to the published contemporary works germane to the controversy, there also survives an amazing wealth of manuscript material. At the outset, Rudwick reminds us of Walter Cannon's observation "that for the middle of the nineteenth century we possess the most complete documentation, for selected individuals, not only that ever has existed but that ever will exist". Certainly much that then achieved semi-permanence through committal to correspondence is today lost down the mouthpiece of Alexander Graham Bell's invention, and the kind of detailed analysis here achieved by Rudwick will for our own century probably prove quite impossible.

Recently, a number of writers — Rudwick amongst them — have emphasized that many nineteenth-century British geologists perceived their activities in terms of a military campaign designed to force nature into a revelation of the sec-

rets of her history. The analogy might be extended to Rudwick himself. He is like a military historian seeking to understand the true nature of some conflict of arms through a detailed study of platoon diaries, squadron workshop records or the signal logs of participating warships. But his object is not merely to trace the course of one particular battle; he seeks to draw lessons of significance for all those involved with conflicts in science both past and present.

Any adequate précis of so detailed and closely reasoned a work is impossible. It suffices to observe that its theme is the argument that occurred in the 1830s and 1840s over the character of the stratigraphy and structure of Devon, but, as Rudwick amply demonstrates, this was no local squabble; the debate had international ramifications extending from Russia into the New World. The protagonists were the geological giants of the day — men such as De la Beche, Lyell, Murchison and Sedgwick — and at stake were geological issues such as the truth of the doctrine of characteristic fossils, the possibility of coal-seams existing within the greywacke and the relative importance of field evidence as opposed to theory-based conclusions. At a more human level we encounter personal jealousies, priority disputes,

back-room lobbying, the suppression of one paper and the slurring of adverse field evidence in others, charges of professional incompetence and an obsessive sense of territoriality by individuals for their own field ground. In addition new light is shed upon the origin of the Silurian System and, of course, upon the birth of the Devonian System itself.

In writing the book Rudwick has enjoyed two great advantages. First, he is himself an experienced geologist sympathetic to the problems faced by those who aspire to understand rocks in the field. He knows all about rains that convert field-sheets to a pulp and about stream sections where the rocks disappear beneath the drift overburden at some vital juncture. Such a sympathy is often wanting in those who approach the history of the earth sciences via history rather than geology. Indeed, Rudwick specifically informs us that he and his wife travelled the lanes of Devon seeking "clues to nineteenth-century perceptions of its geology". Secondly, he has found that for which every author prays: an indulgent publisher. Rudwick has been allowed to present his study upon a most lavish scale. There is no hint of quibbling over wordage or penny-pinching over illustration. The introductory material occupies no less than 60 pages and the ambience of the work as a whole is reminiscent of a PhD dissertation produced by an enthusiastic student who has chosen to disregard the regulations of the Graduate Studies Committee. Sir Peter Medawar has recently declared "that nearly all books on nearly all subjects — especially the philosophical — are much too long"; whether Rudwick should have been encouraged to greater brevity can only be a matter for opinion.

Important though the book is, prospec-



Caricature of controversy — "Preconceived Opinions v/ersus Facts", De la Beche's cartoon of the scene at the Geological Society (December 1834) when his report of fossil plants in the Greywacke of Devon was criticized by Murchison and Lyell.

[De la Beche:] This, Gentlemen, is my Nose.

[His Critics:] My dear Fellow! — your account of yourself generally may be very well, but as we have classed you, *before we saw you*, among men *without noses*, you cannot possibly have a nose.

From *The Great Devonian Controversy*

tive readers may be warned that they will find much of it to be somewhat indigestible. The very scale of the feast is itself daunting as course follows course, and long sections — especially in Part 3 which analyses the forces responsible for shaping our natural knowledge — make very considerable demands upon the reader's powers of concentration. Only an insomniac is likely to select the tome as bedtime reading. Rudwick explains that he had three classes of reader in mind: sociologists and philosophers of science; historians of science; and practising geologists. The first two of these will neglect the book at their peril; Rudwick adduces from his study many lessons which have wide applications in areas of science far removed from those here discussed.

Whether he will be able to win and hold his third class of reader — the geologist — is less certain. He hopes that his book will encourage geologists to reflect upon the nature of their own research activity and he certainly invites them to contemplate some fascinating matters. Not the least of these is the question of the relationship between the human construct of a Devonian System and the actual character of events as they occurred upon the surface of the Earth hundreds of millions of years before the appearance of *Homo sapiens*. In short, in Rudwick's own words, what is the reliability of the Devonian System "as a representation of a portion of a real past history of the earth"? Few geologists will ever have been confronted by so startling and disconcerting a question but surely a work built upon a rather less grandiose scale than this one would have offered far more hope of capturing the attention of practising earth scientists and of turning them momentarily aside from their electron microprobes and X-ray diffractometers.

Rudwick concludes his story at the somewhat unsuccessful Cork meeting of the British Association held in August 1843, but an Irish geologist must observe that had Rudwick chosen to linger awhile amidst the rocks of southern Ireland he might have added one final twist to his tale. In the 1860s Professor J. B. Jukes, the Director of the Geological Survey of Ireland, became convinced that in southern Ireland he had discovered a unique key to the rocks of Devon and he sought to have re-opened the entire Devonian issue. Jukes was one of the finest field geologists of his generation but his one-man campaign had achieved little by the dawn of the day in early May 1869 when he found himself committed to a Dublin lunatic asylum. There Jukes died two months later. That bizarre little episode now awaits the attention of some scholar eager to erect an outhouse to the rear of Rudwick's splendid Gothicedifice. □

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The trouble with book making

Colin Patterson

Oxford Surveys in Evolutionary Biology. Volume 1: 1984. Edited by R. Dawkins and M. Ridley. *Oxford University Press: 1984 (1985).* Pp. 227. £25.

THIS is an enigmatic publication. Though it looks like a book, with hard cover and dust jacket, it is the first issue of a new serial. Though new, the only introductory or explanatory matter lists a 15-strong editorial board which does not include one of the two editors. Though edited and published in Oxford, UK, all 11 contributors are American.

Given no introduction or policy statement, the reader must reconstruct the aims and scope of the *Surveys* from the contents, seven papers and a book review supplement. The papers are: J. T. Bonner on chemical signal-receptor systems; Lynn Margulis and Dorion Sagan on the origins of sex and its maintenance not as adaptation but as "imperative relic"; J. E. Lloyd on deception in nature, particularly in firefly flashing; D. H. Janzen on sphingids and saturniids as alternative ways of being a big moth; O. T. and D. J. Solbrig on plant growth hierarchies and fitness; M. T. Clegg on multilocus population genetic theory and practice; and Niles Eldredge and S. N. Salthe on hierarchy and evolution, a shot at outlining a theory more complete than neo-Darwinism. Finally, M. T. Ghiselin reviews "nine books of cladism" — "on" is intended here, and the books reach back to the 1970s, though one might miss the fact since the publication date of the oldest is omitted.

Of course, that last comment is carping over trivia, but misprint, anachronism and omission are unfortunately typical of the slapdash production of this book. For example, mismatches between text citations and bibliographies are rife — in one paper 26 per cent of the references mentioned in the text are inconsistent with the bibliography. The same bibliography contains 1985 publications along with 1983 papers cited only as "in press", without title or journal. And Daniel Janzen's fascinating essay is marred by execrable printing of the four colour plates.

At 55 pages Janzen's paper is the longest and has the strongest empirical content of any in the book, but that content is not to be retrieved from the index, which is formed on some idiosyncratic principle by which certain papers are indexed in excruciating detail, but Janzen's is found under only three headings (the name of the Costa Rican park where the work was done and the names of the two moth families, one of which is misspelled). While the general tone of most of

the papers is panslectionist, or organismic rather than molecular, the index gives precisely equal space to genetic drift and natural selection, one page reference each. In short, the index is untrustworthy and so useless.

But what about the papers? Well, they vary greatly in what they demand of the reader; some are original; most are conspicuously well written. I hope that others may appreciate their quality without the mounting irritation over production faults that spoiled my reading. If this new serial is to make itself a niche among the journals and the various *Annual Reviews* that cover the burgeoning field of evolutionary biology, someone will have to take a lot more trouble over it. □

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On scale

E. J. Aiton

Measuring the Universe: Cosmic Dimensions from Aristarchus to Halley. By Albert Van Helden. *University of Chicago Press: 1985.* Pp. 203. \$30, £27.50.

ALTHOUGH the generally accepted scheme of planetary distances remained virtually unchanged throughout the Middle Ages and up to the time of Copernicus, it is only within the past 20 years that Ptolemy has been shown to have been its author. Following a surmise of Willy Hartner that the planetary distances were contained in a missing part of Book II of Ptolemy's *Planetary Hypotheses*, Bernard Goldstein located the scheme in a section of an Arabic version of Book I which has not survived in Greek.

The story of man's endeavours to assess the scale of the Universe goes back to the third century BC and the attempts of Aristarchus to measure the sizes and distances of the Sun and the Moon. His methods of lunar dichotomy and eclipse observations, though mathematically sound, depended on an accuracy of measurement which is impossible to achieve even today. As for the distances of the planets and stars, Ptolemy, writing in the second century AD, could only appeal to a system of space-filling spheres that could accommodate his eccentrics and epicycles.

In his study of the evolution of thought on cosmic dimensions, Albert Van Helden offers a clear and informative analysis of the sources and also the findings of modern scholarship relating to an important aspect of astronomy which has not hitherto been the subject of a separate monograph. He sees the invention of the telescope as a turning point in the path leading from the philosophically based scheme of Ptolemy to the determination

of reasonably accurate measured distances and sizes in the latter part of the seventeenth century.

The system of Copernicus not only permitted the deduction of the relative distances of the planets from the geometrical models but also revealed gaps between the spheres. Kepler explained the gaps by means of his polyhedral hypothesis and constructed a harmonic theory of the relative distances which agreed remarkably well with the empirical results based on Tycho's observations and his own planetary laws. Kepler broke with tradition in greatly enlarging the solar distance, though he kept it as small as possible; his analysis of the parallax of Mars and his calculations of eclipses had convinced him that the solar parallax could not exceed 1'. By adopting this value, he still underestimated the absolute size of the planetary system by a factor of about six. While the telescope had brought the possibility of determining the apparent diameters of the planets, the poor optical quality and the technical difficulties of observing prevented accurate measurements, so that Kepler had to combine harmonic hypotheses with observations in his derivation of their sizes. Soon after Kepler's death, the seemingly paradoxical smallness of Mercury revealed by observations of the transit in 1631 demonstrated the need for a new set of apparent diameters based entirely on telescopic measurements.

Accurate determination of the planetary distances and sizes was eventually achieved through the application of new technical inventions, namely the micrometer and telescopic sight, a better understanding of refraction and improved solar theory. Prominent among the astronomers who pursued these investigations were Flamsteed in England and Cassini in France. The popular view of historians and astronomers that the first successful measurement of the parallax of Mars (and hence of the solar parallax) resulted from the simultaneous observations made by Cassini in Paris and Richer in Cayenne in 1672 is contested by Van Helden, who explains that the error margin was too high to justify great confidence in the results obtained. Though producing values fortuitously close to those accepted today, the method employed was severely criticized by Halley, who advised that, for an accurate determination of the solar distance, astronomers would have to await the transit of Venus in the next century. Nevertheless, the publication by Flamsteed and Cassini in 1673 of a value for the solar parallax of 9.5" or 10" (equivalent to a solar distance of about 88 million miles) is seen by Van Helden as a watershed in the measurement of the cosmic dimensions. □

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Business of biology

Renton Righelato

Reshaping Life: Key Issues in Genetic Engineering. By G. J. V. Nossal. Cambridge University Press: 1985. Pp.158. Pbk £6.95, \$11.95.

Setting Genes to Work: The Industrial Era of Biotechnology. By Stephanie Yanchinski. Viking: 1985. Pp.157. £12.95.

The Gene Factory: Inside the Biotechnology Business. By John Elkington. Century, London: 1985. Pp.240. £12.95.

"WHEN historians look back on the twentieth century they will conclude that its first half was shaped by the physical sciences but its second by biology." So begins one of these three new popular books describing industrial biotechnology and the potential benefits of the applications of molecular biology. All three are short, easy to read and suitable for the educated layman and scientists outside the subject

ing this growing technological power; the author concludes that there is adequate awareness of risks in both the scientific community and the public at large, and that the existing legislative and voluntary frameworks for control are satisfactory. Nossal's particular achievement in this book is that he succeeds in being at once sound and visionary.

The other two books both deal with the commercial development of biotechnology and both are by journalists. In *Setting Genes to Work* Stephanie Yanchinski describes, in an easy style, an industrial revolution based on microbes. She touches lightly on the applications, the companies and the people involved in some of the glamorous uses of biotechnology, largely in medicine, but also in food, agriculture and even computing (a DNA-coded, self-assembling biological computer).

John Elkington, the editor of *Biotechnology Bulletin*, reviews the growth of industrial biotechnology from the late 1960s to the end of 1984. He pieces together short notes from interviews and press re-

Milestones in the commercial development of biotechnology*

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|--|---|
| 1973: First gene cloned. | approved for use in the USA; initial public offering by Cetus sets Wall Street record for the largest amount of money raised in an initial public offering (\$125 million). |
| 1974: First expression of a gene cloned from a different species in bacteria. | |
| 1975: First hybridoma created. | |
| 1976: First firm to exploit recombinant DNA (rDNA) technology founded in the USA (Genentech). | 1982: First rDNA animal vaccine (for colibacillosis) approved for use in Europe; first rDNA pharmaceutical product (human insulin) approved for use in the USA and UK. |
| 1978: First product made with rDNA (somatostatin). | |
| 1980: <i>Diamond v. Chakrabarty</i> —US Supreme Court rules that microorganisms can be patented under existing law; initial public offering by Genentech sets Wall Street record for fastest price per share increase. | 1983: First plant gene expressed in a plant of a different species. |
| 1981: First monoclonal antibody diagnostic kits | 1984: Judge John Sirica thwarts the scheduled environmental release of rDNA organisms; Stanford awarded Cohen/Boyer patent on basic rDNA process. |

* Here abridged, the details are taken from Marc Lappé's *Broken Code: The Exploitation of DNA*, recently published by Sierra Club Books, San Francisco, price \$17.95. The original source was *Commercial Recombinant DNA: An International Analysis*, produced by the Office of Technology Assessment, January 1984.

areas directly involved. The authors each succeed in being up to date, but the subject is changing so rapidly that one feels that each volume should bear a "read by" date.

The least ephemeral of the books is Dr Nossal's, *Reshaping Life*, whose introduction I quote above. He goes on to explain simply, but accurately, the key features of molecular and cell biology, and exemplifies their practical application. These early chapters on cellular organization and the genetic code can be skipped by those with a basic biological training.

There follows a discussion of a number of actual achievements and exciting potential uses, among them genetically engineered proteins for correction of metabolic disorders and new vaccines, gene therapy and gene probes in diagnosis — developments which will fundamentally effect the quality of life and longevity. Whilst health-care applications dominate, there is a section on the uses of genetic engineering in agriculture and the food industry, and another describing the growth of biotechnology companies. Two chapters give a balanced discussion of the ethical and public-policy issues surround-

ports with accounts of most of the British, and many of the American, companies involved in bringing in the newer products which depend on genetic engineering and other aspects of biotechnology. Accuracy is sometimes sacrificed to style, but as a catalogue of commercial developments it is readable and reasonably comprehensive: over 200 companies are mentioned, concerned with the applications of biotechnology in medicine, agriculture, animal breeding and food production.

In both Yanchinski's and Elkington's books I would have liked a more critical analysis, perhaps through case studies, of the business and technical factors which have made ventures succeed or fail. But, perhaps we should be grateful that those with the scientific and commercial acumen to write such a book are too busy with their demanding task of developing the products and building the new businesses on which the reality of our much-heralded revolution depends. □

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Never ending circuits

Peter Beverley & Andrew McMichael

The Biology of Idiotypes. Edited by Mark I. Greene and Alfred Nisonoff. Plenum: 1984. Pp.507. \$59.50, £56.53.

Idiotypy in Biology and Medicine. Edited by Heinz Köhler, Jacques Urbain and Pierre-André Cazenave. Academic: 1985. Pp.445. \$78, £78.

It is more than twenty years since immunoglobulin idiotypes were first described by Oudin and Kunkel, and more than ten since Niels Jerne proposed his theory of an idiotypic-based immunoregulatory network. The recent award of a Nobel prize to Jerne is a recognition of the importance of his contribution, though many of the key observations were made before he proposed his theory and before the modern techniques of protein chemistry and molecular genetics became available.

After the initial description of specific antigenic determinants (idiotypes) present on antibody molecules raised against bacterial antigens, it was shown that the same idiotypes could also be detected on immunoglobulin unreactive with the immunogen. In addition idiotypes were shown to be under genetic control. None of this linked idiotypes to immunoregulation but the network theory soon did so. Supporting experimental evidence rapidly appeared, perhaps the earliest being the observation that (auto) anti-idiotypic is produced during the course of a normal immune response. This theoretical and experimental base stimulated an enormous number of experiments which are the subject of the two books reviewed here. Two major topics are covered: the structural basis of idiotypes, and their role in regulation of immune responses in health and disease. The more practical question of whether anti-idiotypic responses can be used to control aberrant immune responses underlies much of the thinking. None of these issues has yet been resolved but the attempts to do so

make interesting and instructive reading.

Of the two books, *The Biology of Idiotypes* is the more biochemical, and *Idiotypy in Biology and Medicine* is more concerned with networks and their role in disease. There is, however, considerable overlap between them, with several authors contributing to both.

The first half of *The Biology of Idiotypes* is devoted to the molecular, genetic and structural aspects of idiotypes, with several chapters presenting data on the expression of private and cross-reacting idiotypes on families of antibodies reacting with a simple, structurally defined antigen. These chapters illustrate the power of the hybridoma method for producing antibodies of defined specificity in mice of any desired genetic makeup, whereas previous work on the structural basis of idiotypy relied upon myeloma paraproteins, often of unknown antigen specificity and uncontrolled genetic origin. Nevertheless, in spite of the sequence data on families of related, defined-antigen-binding, monoclonal antibodies, there are still very few examples where a particular amino acid residue can be definitively associated with expression of an idiotypic determinant. (A new and intriguing example was described recently by Radbruch *et al.* in *Nature* 315, 507 (1985), where an amino acid change in the diversity (D) segment of the antibody led to loss of idiotypy but not antigen specificity.) The reasons for this are made clear. Most antibodies, including anti-idiotypes, react with conformational determinants made up of amino acids which may be widely separated in terms of primary sequence. From the genetic point of view analysis is complicated by the observation that contributing to variability are not only variable region (V) genes, but also D and joining region (J) minigenes, as well as infidelity of VDJ assembly and somatic mutation. All of these issues are extensively and well discussed, as is the limited amount of information on the three-dimensional structure of immunoglobulin molecules, which, combined with sequence analysis, may ultimately hold the key to understanding the structural basis of idiotypes.

The second half of *The Biology of Idiotypes* and most of *Idiotypy in Biology and Medicine* deals with the regulation of idiotypes. The network theory itself is particularly well discussed in the latter book, by Köhler, and there are detailed accounts of experiments designed to test its validity. These sections are very much for cellular immunologists, covering cell transfer experiments, separation of T and B cells, induction of help and suppression and experimental immune diseases. Many of the experiments described exemplify the powerful effects which administration of idiotypes or anti-idiotypes can have on subsequent immune responses.

The problem with these chapters is that they have been overtaken by events of the

past two years. Several contributors were wrestling with the difficulty of integrating T and B cells into a coherent network at a time when the T cell receptor had not been characterized. The ease with which T-cell idiotypes have subsequently been detected by monoclonal antibodies, and the general similarity of the T-cell receptor chains to immunoglobulin light chains, now make an integrated network much more credible. However, it is still not clear that T-cell receptors can respond to idiotypes on B cells or other T cells. In Köhler's reiteration of Jerne's view, the T-cell repertoire may be selected and expanded by the images of B-cell antibody receptors presented with self-antigen of major histocompatibility complex (MHC). This neatly explains the apparent genetic linkage between the two, a finding that can now be tested with much greater rigour.

Another question is whether or how to fit in antigen processing. Although not universally accepted, there is considerable evidence that helper T cells respond to processed (fragmented) antigens plus MHC Class II antigens. Are they able therefore to recognize idiotypic processed and presented on T or B cells in a manner similar to other antigens? Although not known for T cells, B cells can clearly present foreign antigen. But are the rules different for self idiotypes (and allo-MHC antigens)? Should we be looking for idiotypic determinants which are the same as those seen by B cells, or at short, linear amino acid sequences presented with the self-MHC antigen?

Further, there is another layer of complexity: the antigens could select the T cells in a positive fashion; it is known that the antibody to the T-cell antigen receptor can stimulate T-cell division, as can antibody to the associated CD3 glycoprotein. One experiment, described by Greene *et al.* in *Idiotypy in Biology and Medicine*, indicates that a B-cell hybridoma secreting anti-idiotypic specific for anti-reovirus $\sigma 1$ glycoprotein is killed by cytotoxic T cells specific for the same antigen. We need to know who recognizes whom.

These issues can now all be addressed and the appropriate experiments are surely in progress. For many readers, ultimate proof of the network hypothesis will depend upon clear understanding of the interactions between the receptors of T and B cells and between those of the T cell subsets. The two books contain many interesting data and ideas, and can be thoroughly recommended to those who wish to prepare themselves for these impending and, we can hope, conclusive advances. □

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New in paperback

• *The Nature of Mathematical Knowledge* by Philip Kitcher. Publisher is Oxford University Press, price is £6.95, \$8.95. For review see *Nature* 307, 189 (1984).

• C. Kittel's classic textbook *Quantum Theory of Solids*, which first appeared in 1963. Publisher is Wiley, price is \$21.95, £21.95.

On disc

A.N. Barrett asks us to point out that the price of his book *Mathematics, Biology and Microcomputers* (reviewed in *Nature* 314, 476; 1985) includes a floppy disc on which are included all the programs listed in the book.

The reference to prime number technique ("in press" in the book) is *Int. J. bio-med. Comput.* 16, 149-155 (1985).

Limiting and realizable efficiencies of solar photolysis of water

James R. Bolton*, Stewart J. Strickler† & John S. Connolly‡

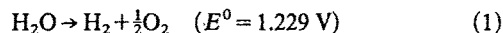
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A critical analysis of various schemes for the solar photolysis of water into hydrogen and oxygen places ideal limits on their efficiencies. Single-bandgap schemes will have practical efficiencies of up to 10%, but the prospects of higher efficiencies from dual-bandgap schemes are encouraging.

OF the many reactions proposed for solar generation of energy-rich reduced molecules (fuels) using common reagents such as water, carbon dioxide and nitrogen¹, none has gained as much attention as the solar photolysis of water



Because water is transparent to virtually all of the solar spectrum, sensitizers must be used if sunlight is to drive the reaction. Heidt² first reported a complete water-photolysis reaction; in his system the reaction was sensitized in low yields by Ce^{4+} and Ce^{3+} using ultraviolet light. Fujishima and Honda³ later reported the first complete photoelectrolysis of water at semiconductor electrodes. Since then, many sensitizers and water-decomposition systems have been studied (see refs 1, 4–20 for reviews).

In the light of recent interest in the reaction, it is important to examine limits on its efficiency and, further, to estimate efficiencies that might be achieved based on realistic assumptions. This problem was examined in 1978 (ref. 1) in the general context of solar fuel-generation reactions. Here, we update this study and specifically analyse the water-photolysis reaction.

Classification of reaction schemes

Balzani *et al.*⁸ were the first to present an extensive analysis of various schemes for solar water decomposition. They proposed four cycles, C1–C4, to classify various reaction schemes based on photosensitized processes with successively lower threshold energies needed to initiate the photochemical step. In the C1 and C4 cycles, two photons are required to split one H_2O molecule, whereas in the C2 and C3 cycles one photon per H_2O is required. Only the C3 and C4 cycles lead to formation of molecular H_2 and O_2 . This classification was later adopted by Bolton¹, who added a C5 cycle which involves two photosystems operating at the same threshold wavelength to drive the oxidation and reduction steps, respectively; in this scheme four photons are required to split one H_2O molecule into H_2 and O_2 . Here we have chosen a simpler and broader classification.

There are two critical factors in evaluating schemes for the photolysis reaction: (1) the number of photosystems used to carry out the photochemical process (we consider here mainly schemes using a single (S) photosystem or using dual (D) photosystems with different threshold wavelengths and placed one behind the other); (2) the number of photons used for each hydrogen molecule evolved (we consider the use of 1, 2 or 4 photons). The possible schemes are accordingly denoted S1, S2, S4, D2 and D4 (Table 1).

Ideal limits on efficiencies

Several authors have treated the general problem of ideal limits on conversion of solar energy into useful work or stored chemical energy^{21–28} and there is now general agreement on the significance of these limits on direct solar energy conversion processes. Briefly, three basic approaches have been taken: (1) Shockley

and Queisser²¹ developed the concept of the ideal photovoltaic cell and demonstrated that the maximum conversion efficiency is ~31% in AM0 sunlight. (The intensity of sunlight is reduced by passage through the atmosphere, and the amount of air traversed is an important parameter. Air Mass one (AM1) corresponds to sunlight having passed vertically through the atmosphere to sea level. AM0 refers to sunlight above the atmosphere and AM1.5 to light coming at such an angle (~48° from the vertical) that it has traversed 1.5 times as much air as AM1 radiation.) (2) Ross^{22,23} and Ross and Calvin²⁴ generalized the Shockley and Queisser treatment to all photochemical solar energy conversion processes. Ross and Hsiao²⁵ treated the problem further using a chemical thermodynamic approach and also obtained an ideal efficiency limit of ~31%. (3) Haught²⁶ developed a general analysis based on Planck's and Kirchhoff's laws, and also obtained an ideal limit efficiency of ~31%.

Bolton, Haught and Ross²⁷ then showed that these three approaches are, in fact, equivalent. Porter²⁸ has recently presented a parallel analysis of the problem from the standpoint of successive efficiency losses. We have performed our calculations within the framework of the Ross treatment^{22–27}.

The essential features of the model are as follows: light is absorbed by a material (for example, a semiconductor or a molecular ensemble) having a certain excited-state energy U_g with a corresponding bandgap or threshold wavelength λ_g . Photons of longer wavelength are not absorbed; those of wavelengths shorter than λ_g may be absorbed, but thermal equilibration of the excited state with the surroundings usually occurs very rapidly so that the system relaxes to an energy close

Table 1 Classification system for solar water photolysis schemes

Scheme classification	No. of photo-systems	Minimum no of absorbed photons per H_2	Reaction
S1	1	1	$\text{H}_2\text{O} \xrightarrow{1h\nu} \text{H}_2 + \frac{1}{2}\text{O}_2$
S2	1	2	$\text{H}_2\text{O} \xrightarrow{2h\nu} \text{H}_2 + \frac{1}{2}\text{O}_2$
S4	1	4	$\text{H}_2\text{O} \xrightarrow{4h\nu} \text{H}_2 + \frac{1}{2}\text{O}_2$
D2	2	2	$\text{H}_2\text{O} \xrightarrow{h\nu_1 + h\nu_2} \text{H}_2 + \frac{1}{2}\text{O}_2$
D4	2	4	$\text{H}_2\text{O} \xrightarrow{2h\nu_1 + 2h\nu_2} \text{H}_2 + \frac{1}{2}\text{O}_2$

S, single photosystem; D, dual photosystems operating at different threshold wavelengths λ_1 and λ_2 . Scheme classification can be generalized to the solar production of any reduced fuel by taking the number in column 3 to specify the minimum number of photons required to generate the reducing equivalent of one H_2 molecule.

to U_g within a time <10 ps. We note that, under certain conditions, it is possible that thermal equilibration of the excited states is not achieved before electron transfer occurs; 'hot carrier' effects could, conceivably, then enhance the ideal limiting efficiencies²⁹.

In agreement with the requirements of microscopic reversibility, the excited state can radiate; this limits the concentration of excited states or minority carriers that can be built up. If electrical power or chemical products are to be extracted, there will necessarily be a further reduction in excited-state concentration. The model predicts that the efficiency of conversion of incident solar energy to chemical (Gibbs) energy is limited to

$$\eta = \frac{J_g \cdot \mu_{ex} \cdot \phi_{conv}}{S} \quad (2)$$

where J_g is the absorbed photon-flux (photons $s^{-1} m^{-2}$) for solar wavelengths such that $\lambda \leq \lambda_g$; μ_{ex} is the excess chemical potential generated in the system by absorption of light; ϕ_{conv} is the quantum yield for conversion of absorbed photons into products and S is the total incident solar irradiance ($W m^{-2}$). ($\phi_{conv} = 1.0$ means that every absorbed photon is 100% effective in generating products. Thus, in terms of the classification scheme used here, the corresponding quantum yields of H_2 formation would be 1.0 for S1, 0.5 for S2 or D2 and 0.25 for S4 or D4 systems.) The quantities μ_{ex} and ϕ_{conv} are not independent; the highest values of μ_{ex} can be achieved only for small ϕ_{conv} and vice versa. The Ross treatment²²⁻²⁷ allows optimization of these parameters to give an ideal maximum efficiency η_p for any given λ_g . (Strictly speaking, η_p is not a thermodynamic limit in that it is defined for a condition of maximum power and not for a reversible process.)

For an ideal chemical system^{25,27} with-negligible depletion of the ground state

$$\mu_{ex} = \mu_{light} - \mu_{dark} = kT \ln \left(\frac{x_{A^*}}{x_{A^*}^{eq}} \right) \quad (3)$$

where μ_{light} and μ_{dark} are the chemical potentials of A^* in the light and dark, respectively; x_{A^*} is the mole fraction of excited states A^* present at steady state under solar irradiation; and $x_{A^*}^{eq}$ is the corresponding mole fraction present at equilibrium in the dark at absorber temperature T (taken as 300 K). An important concept in the Ross treatment is that there is always a certain fraction of U_g which is unavailable to do work. We term the unused energy per photon U_{loss} , defined by

$$U_{loss} = U_g - \mu_{prod} \quad (4)$$

where μ_{prod} is the chemical potential of the products. Ross has pointed out that the upper limit of μ_{prod} is μ_{ex} , and showed that the optimum efficiency is achieved with $U_{loss} \sim 0.3-0.4$ eV, the exact value depending on λ_g . Details of these calculations are described elsewhere^{25,27}.

To account for losses in the production of solar fuels by real systems, Bolton¹ introduced the factor η_c , which is the overall efficiency of conversion of solar energy to stored chemical energy

$$\eta_c = \eta_g \eta_{chem} \phi_{conv} \quad (5)$$

where η_g is the fraction of incident solar irradiance that is converted to energy of the lowest excited state of the absorber

$$\eta_g = \frac{J_g U_g}{S} \quad (6)$$

η_{chem} is the fraction the excited-state energy converted to stored chemical energy and is defined by

$$\eta_{chem} = \frac{\Delta G^\circ/n}{U_g} = \frac{U_g - U_{loss}}{U_g} \quad (7)$$

and ϕ_{conv} is the quantum efficiency of the fuel-forming reaction. In equation (7) ΔG° is the standard Gibbs energy and n is the number of photons used to drive the reaction as written. In real systems, U_{loss} will be greater than the ideal value of $\sim 0.3-0.4$ eV (ref. 27); the actual value will be determined by the degree to

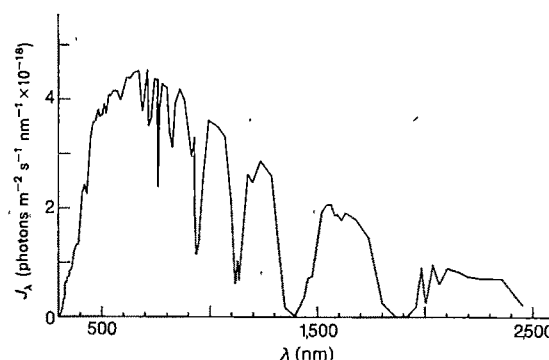


Fig. 1 Spectral distribution of the solar photon flux based on the AM1.5 global data of Bird *et al.*³².

which any real system approaches the ideal limit. In principle, the quantum efficiency factor ϕ_{conv} in equation (5) can be made very close to unity for real systems and is ~ 0.98 for the 'ideal' case²⁷. A major goal of practical systems is to achieve ϕ_{conv} close to unity.

Because the reactions we are considering are endergonic, the entire ΔG° must be obtained from photon energy. Hence, for a given reaction and U_{loss} there will be a threshold wavelength λ_t which is just capable of driving the reaction; specifically as shown by Bolton, Haught and Ross²⁷

$$\lambda_t = \frac{hc}{[\Delta G^\circ/n + U_{loss}]} \quad (8)$$

where h is Planck's constant and c is the speed of light.

Absorbed photon flux

We have shown in equations (2) and (6) that J_g is an important quantity in solar efficiency calculations. J_g is defined by²⁷

$$J_g = \int_0^{\lambda_g} J_\lambda \alpha(\lambda) d\lambda \quad (9)$$

where $\alpha(\lambda)d\lambda$ is the fraction of incident radiation absorbed in the wavelength band from λ to $\lambda + d\lambda$, and J_λ is the corresponding incident spectral solar photon flux (photons $m^{-2} s^{-1} nm^{-1}$). The solar spectrum is conventionally measured as a spectral irradiance E_λ ($W m^{-2} nm^{-1}$) which is related to J_λ by

$$J_\lambda = \frac{E_\lambda \lambda}{hc} \quad (10)$$

The choice of an appropriate solar spectral distribution is an important consideration as it is known³⁰ that such distributions vary considerably with changes in atmospheric conditions. However, Buhl *et al.*³¹ have recently shown that if one chooses a 'global' distribution (that is, one including light from all angles incident on a surface) rather than a 'direct beam' distribution, the calculated ideal efficiencies do not vary much with changes in atmospheric conditions (for example, turbidity, water vapour and ozone concentrations) or even air mass. (Global distributions are appropriate for flat-plate collectors, whereas direct-beam distributions are appropriate for concentrating collectors since the diffuse component cannot be concentrated.) Thus, we have chosen to use the unconcentrated, global AM1.5 spectral irradiance distribution developed by Bird *et al.*³², probably the best data currently available. (A printout of I_λ , J_λ and the integrated photon flux J_g for $\lambda \leq \lambda_g$ is available from any of the authors.) A plot of J_λ against λ is shown in Fig. 1.

Analysis of limits

Considered as an oxidation-reduction process, reaction (1) involves a two-electron transfer with a standard cell potential of 1.229 V. Thus, for S1 schemes, 2.458 eV Gibbs energy must be supplied per photon. S2 or D2 schemes require an average of 1.229 eV per photon and S4 or D4 schemes only 0.615 eV per photon. In the following discussion we divide our analysis

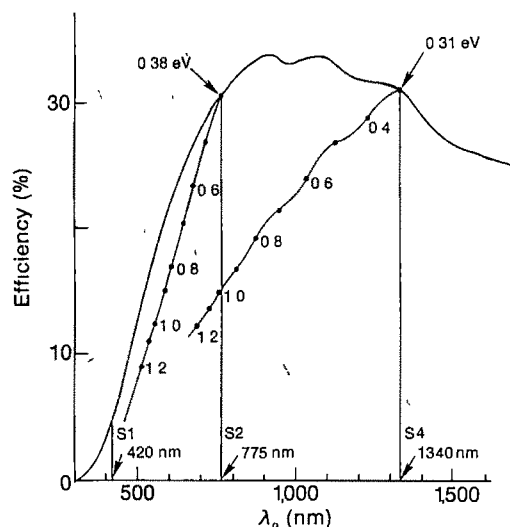


Fig. 2 Efficiencies for conversion of solar radiation to stored Gibbs energy using single-bandgap devices. The full curve shows η_p , the ideal limiting efficiency. The vertical lines at 420, 775 and 1,340 nm show the maximum threshold wavelengths for reaction (1) carried out by S1, S2 and S4 schemes, respectively, under ideal conditions. The descending curves through the dots show the changes in efficiencies and bandgap wavelengths for values of U_{loss} greater than the optimum; the numbers at the dots are the respective values of U_{loss} in eV per photon at those points.

according to whether the sunlight is absorbed in a photosystem with a single bandgap (S) or in a system with dual bandgaps (D). **Single-bandgap systems.** The upper curve in Fig. 2 shows a plot of the ideal limiting efficiency, η_p , against the bandgap wavelength λ_g for the AM1.5 global solar distribution. Similar curves have been calculated for other terrestrial distributions^{1,27,31}. η_p rises sharply in the range 400–800 nm primarily because J_g increases rapidly in this region. However, as λ_g increases, the available Gibbs energy per photon decreases; for $\lambda_g > 1,100$ nm this causes η_p to decrease. The curve in Fig. 2 shows the ideal limit on the efficiency of any single-bandgap device for direct (that is, quantum) conversion of unconcentrated sunlight to chemical or electrical energy. If the respective optimum values of U_{loss} are inserted into equation (8), it is possible to calculate the 'ideal' values of λ_t for S1, S2, or S4 water-photolysis schemes. These wavelengths are indicated in Fig. 2 by vertical lines along with the corresponding values of U_{loss} . It is clear from the figure that S1 schemes can be removed from further consideration for practical systems because even the 'ideal' maximum efficiency for an S1 system is only ~5%. Thus, the remainder of the analysis in this section deals only with the S2 and S4 cases.

We first consider S2 systems. In accord with Fig. 2, the ideal maximum efficiency for the S2 case is 30.7% at $\lambda_g = 775$ nm.

(This analysis is based on the standard potential of reaction (1) which assumes that the product gases are produced at 1 atm pressure and at 298 K. If the products are produced at a lower pressure, the Gibbs-energy requirement will be reduced and the threshold wavelength will shift to the red. For example, at a pressure of 10^{-3} atm hydrogen, the potential of the reaction is only 1.052 V; from equation (8), $\lambda_t = 880$ nm, and the apparent maximum efficiency could increase to 33.3%. Of course, if the hydrogen produced must be compressed to ≥ 1 atm, work will have to be expended, so the gain in efficiency obtained by using a longer wavelength threshold could not be fully realized.) Under these ideal conditions, U_{loss} has the optimum value of 0.37 eV. Solar conversion efficiencies for water photolysis under various conditions are collected in Table 2.

It is worthwhile to consider briefly the physical meaning of the various energy losses in a photochemical system. The ideal S2 system would require the use of molecules with excited-state energy $U_g = 1.60$ eV ($\lambda_g \approx 775$ nm); photons with shorter wavelengths will produce excited states of higher energy, but these will relax very quickly to U_g with dissipation of any extra energy into heat. To a good approximation, we can regard 1.60 eV as the standard chemical potential of the excited state relative to that of the ground state. But the concentration of the excited state will be much less than that of the ground state, so its actual chemical potential relative to the ground state, given by equation (3), will be lower than the standard value. Under optimum conditions, it will be lower by 0.37 eV, giving a value of $\mu_{\text{ex}} = 1.23$ eV, just enough to drive reaction (1) if there are no additional losses. The energy difference $U_{\text{loss}} = 0.37$ eV is required to be delivered as additional heat to the surroundings when the excited states are returned to the ground state in the process of extracting work to carry out the chemical reaction. The complete derivation of the optimum U_{loss} will be found in earlier literature^{1,27}.

Real systems clearly will involve U_{loss} greater than the ideal value. For example, in a photoelectrochemical cell the electrode polarization (or overvoltage) will contribute to U_{loss} , as this term must include all polarization losses, even those at a dark electrode. Figure 2 also shows what happens to the efficiency and λ_t as we assume various larger values of U_{loss} . We feel that a reasonable goal for an operating system would be $U_{\text{loss}} \sim 0.8$ eV, in which case the efficiency could be no more than ~17% at $\lambda_t \sim 610$ nm. As U_{loss} increases beyond this value, the attainable efficiency drops very rapidly (Fig. 2, Table 2). This results primarily from the fact that as λ_t shifts to the blue, the fraction of the solar photon flux absorbed drops very rapidly. Therefore, we conclude that S2 schemes will require sensitizers with $\lambda_g < 610$ nm (or semiconductors with a bandgap of > 2.0 eV) to drive reaction (1). Also, the system will have to be very carefully designed to minimize all contributions to U_{loss} .

The major problem with S2 systems is that they are limited to rather short threshold wavelengths, and, therefore, the absorbed solar photon flux is low. We now consider one way around this difficulty, the use of two photons to drive each

Table 2 Solar conversion efficiencies and other related data for solar photolysis of water using global AM1.5 solar radiation

Scheme	Conditions	η_p (%)	η_c (%)	U_{loss} per photon (eV)	Threshold wavelengths λ_t or λ_1 (nm)	λ_2 (nm)
S1	Ideal limit	5.3	—	0.49	420	—
S2	Ideal limit	30.7	—	0.37	775	—
S2	Chemical conversion	—	23.5	0.60	680	—
S2	Chemical conversion	—	17.4	0.80	610	—
S2	Chemical conversion	—	12.7	1.00	555	—
S4	Ideal limit	30.6	—	0.31	1,340	—
D2	Ideal limit	42.4	—	0.38*	655	930
D4	Ideal limit	41.0	—	0.31*	910	2,610
D4	Chemical conversion	—	32.3	0.60	785	1,465
D4	Chemical conversion	—	27.1	0.80	720	1,120
D4	Chemical conversion	—	21.6	1.00	655	925

In schemes D2 and D4, it is assumed that equal photon fluxes are used by the two photosystems.

* Average U_{loss} per photon.

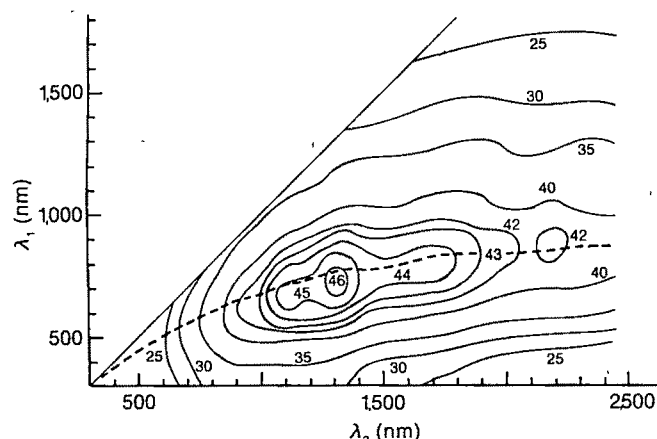


Fig. 3 Contour diagram showing the ideal limiting efficiencies (η_p , %) in a layered dual-bandgap system with bandgap wavelengths λ_1 and λ_2 . Dashed line, path of equal effective photon fluxes used in two photosystems as required for use of the two halves in series.

electron in the reaction, or four photons per H_2 molecule produced. Less energy is required from each photon, a longer threshold wavelength is possible, and so more photons can be absorbed. As indicated in Fig. 2 and Table 2, the ideal S4 case occurs with $\lambda_1 = 1,340$ nm and $U_{\text{loss}} = 0.31$ eV. The ideal maximum efficiency is $\sim 30.6\%$, virtually the same as in the S2 case. However, as U_{loss} is increased above the ideal value, the efficiency does not drop quite as rapidly for the S4 system, and at $U_{\text{loss}} = 0.8$ eV per photon the efficiency would be $\sim 19\%$ (Fig. 2).

Dual-bandgap systems. The dilemma faced by a designer of a single-photosystem process is that if a high λ_g is used so as to absorb many photons, the energy available per photon is low, whereas if a low λ_g is used to give a high energy per photon, few photons can be absorbed. This suggests a scheme using two or more absorbing materials (or photosystems) with different bandgaps, placed one behind the other. The first, with bandgap wavelength λ_1 , is placed on top (that is, towards the Sun) and the other, with a longer bandgap wavelength λ_2 , is placed behind it to use photons not absorbed by the first. The first photosystem is assumed to absorb all photons with $\lambda \leq \lambda_1$, whereas the second absorbs all photons with $\lambda_1 < \lambda \leq \lambda_2$.

We first consider a case in which the two photosystems operate independently; each one is treated with the same equations used for a single photosystem with the respective bandgap and the proper number of photons absorbed. For each absorber we calculate the ideal maximum efficiency and then add the two values to get the total possible efficiency for the system. The results are displayed in Fig. 3 in the form of a contour diagram, showing the ideal limiting efficiencies as a function of the two bandgap wavelengths. Similar diagrams have been presented by Ross and Hsiao²⁵ and by Bolton¹. There is a considerable increase in the limiting efficiency compared with a single photosystem. The maximum solar efficiency (η_p) is $\sim 46\%$; this occurs when the top layer absorbs photons of wavelength shorter than ~ 720 nm and generates a chemical potential of ~ 1.34 eV, while the second layer absorbs the somewhat larger number of photons available with wavelengths between ~ 720 and $\sim 1,320$ nm and generates a chemical potential of ~ 0.61 eV. The two layers presumably would be used to drive two different uncoupled processes.

An alternative and attractive scheme for using two-layer systems is to couple the layers in series to increase the voltage in a photovoltaic device or the chemical potential in a photochemical or photoelectrochemical device. For example, in a photoelectrochemical cell the top layer might be a photoanode to produce O_2 from aqueous solution, while the back absorber would be a photocathode to produce H_2 . This permits the chemical potentials produced by the two sensitizers to combine

and drive a chemical reaction of higher energy than either material could drive alone. Such devices have the important additional restriction that each layer must use the same number of photons, that is, the number of photons absorbed multiplied by ϕ_{conv} must be the same for each layer. We use the optimum value of ϕ_{conv} for each system, which maximizes the energy stored^{1,22-25}. The restriction of equal numbers of photons used defines a relationship between the two bandgaps: essentially the number of photons with $\lambda \leq \lambda_1$ must equal the number with $\lambda_1 < \lambda \leq \lambda_2$, with a small correction for the different quantum yields required for maximum efficiency. This relationship then defines a path across the contour diagram of Fig. 3 as shown by the dashed line. Fortunately, the restriction of equal photon fluxes is not too serious, as the path follows quite closely the ridge of high efficiency across the diagram.

Figure 4 shows a similar contour diagram for solar efficiencies of dual-bandgap systems with an average U_{loss} of 0.8 eV per photon, which we take as a reasonable goal. (Even for larger U_{loss} values ϕ_{conv} can, in principle, be very close to unity. We have used $\phi_{\text{conv}} = 1.0$ to illustrate the maximum efficiencies for a given value of U_{loss} .) As in Fig. 3, the dashed line shows the path of equal photon fluxes absorbed in each layer. It is encouraging to note that this path still follows closely the ridge of high efficiency. The maximum efficiency on this diagram is $\sim 28\%$. The limits for water splitting are indicated and have efficiencies of ~ 24 and $\sim 27\%$ for D2 and D4 schemes, respectively.

Figure 5 presents data from these calculations in a manner which allows a comparison with Fig. 2. The top curve of Fig. 5 shows the ideal limiting efficiencies along the path of equal used photon fluxes plotted as a function of λ_2 ; this curve is the contour along the dashed line of Fig. 3. A few values of λ_1 are marked for reference. The two vertical lines show the respective threshold wavelengths for water splitting by D2 systems ($\lambda_1 = 655$; $\lambda_2 = 930$ nm) and by D4 systems ($\lambda_1 = 910$; $\lambda_2 = 2,610$ nm). Values of U_{loss} greater than the optimum values give a family of efficiency curves for η_c lying below the η_p curve. Three examples are shown with $U_{\text{loss}} = 0.6, 0.8$ and 1.0 eV.

By further analogy with Fig. 2, we also show in Fig. 5 lines representing the threshold wavelengths and efficiencies for water splitting when the energy losses per photon are larger than the ideal optimum values. As before, the threshold wavelength λ_2 shifts to the blue and efficiencies drop rapidly as larger losses are imposed. λ_1 also shifts to the blue; for example, in the D4 case with $U_{\text{loss}} = 0.8$ eV, $\lambda_1 = 720$ nm and $\lambda_2 = 1,120$ nm. Note, however, that because the ideal limiting efficiencies are rather

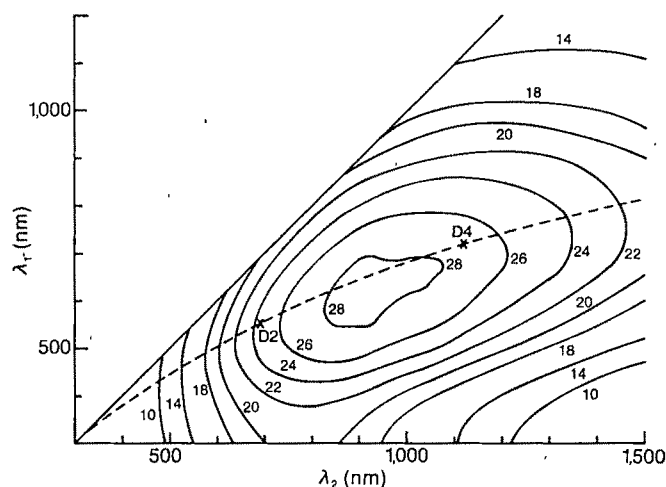


Fig. 4 Contour diagram showing efficiencies for layered dual-bandgap devices with U_{loss} taken as 0.8 eV per photon. By analogy with Fig. 3, the dashed line shows the path of equal photon fluxes absorbed in the two photosystems; the threshold wavelengths are indicated for carrying out reaction (1) with schemes D2 and D4, respectively.

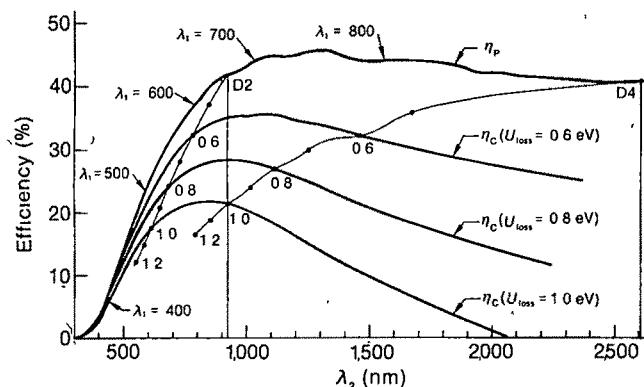


Fig. 5 Conversion efficiencies for dual-bandgap devices with equal photon fluxes used in the two photosystems. By analogy with Fig. 2, the top curve shows the ideal limiting efficiencies (η_p), plotted as a function of λ_2 , with a few values of λ_1 indicated for reference. Vertical lines indicate maximum threshold wavelengths for reaction (1) carried out by schemes D2 and D4 under ideal conditions. Lower curves represent chemical conversion efficiencies η_c for $U_{\text{loss}} = 0.6, 0.8$ and 1.0 eV, respectively, assuming $\phi_{\text{conv}} = 1.0$ in each photosystem. Descending curves through the dots show the threshold wavelengths and efficiencies for U_{loss} greater than the respective ideal optimum values.

high for dual-bandgap systems, efficiencies can remain reasonable even when the losses are appreciable, and the λ_g values fall in the range of available sensitizers.

We emphasize that our analysis of D2 and D4 systems assumes that the second photosystem is placed behind the first to use those photons not absorbed by the first. One can also construct devices with two different photosystems placed side by side. The efficiency of such an arrangement can be analysed by treating each as a single photosystem and taking an area-weighted average of the two efficiencies. Because such systems offer little or no efficiency advantage over single photosystems, we will not analyse them further.

Examples

In this section we mention a few examples to illustrate the possibilities for solar energy conversion.

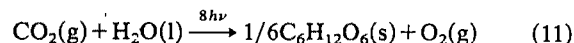
A photoelectrochemical cell using SrTiO_3 as a photoanode and a platinum cathode is capable of carrying out reaction (1) with a quantum yield of nearly unity³³⁻³⁵. However, U_g for SrTiO_3 is ~ 3.2 eV, so it absorbs only ultraviolet light and U_{loss} is ~ 2.0 eV. With this high value of U_{loss} , the solar efficiency is only $\sim 1\%$.

Grätzel^{11,12} and Lehn¹⁶ and their co-workers have reported success in producing both H_2 and O_2 in a photochemical system using trisbipyridyl ruthenium(II) as a sensitizer, methyl viologen as a charge relay and colloidal particles to catalyse the evolution of H_2 and O_2 . This sensitizer absorbs light at $\lambda \leq 500$ nm, so $U_{\text{loss}} = 1.2$ eV. If the quantum yield were high, this U_{loss} would allow an $\sim 8\%$ solar conversion efficiency. However, the quantum yield is itself only $\sim 5\%$, so if used as a solar converter, this system would have an actual efficiency well under 1% . In our analysis of limits, we have generally assumed the quantum yield to be high; this example serves to point out that this may not always be the case. One of the major tasks in designing practical systems is to devise ways to make the quantum yield approach unity. Both of the above cases are examples of S2 systems.

The Texas Instruments company has patented a system³⁶⁻³⁸ for splitting HBr rather than H_2O , using two sets of micro-spherical silicon photovoltaic arrays in series, with metal and metal oxide catalytic electrodes. This is not a true photoelectrochemical device but rather a tandem, photovoltaic-electrolysis system³⁹. The two sets of photovoltaic elements are slightly different but have the same λ_g , and are arranged side by side. The S4 analysis presented above applies to the system. Solar-to-chemical conversion efficiencies of ~ 4.5 – 5.5% have

been achieved for cell areas in the range of 100 – $1,000$ cm^2 (ref. 38). The bandgap energy of silicon is 1.1 eV, but it is an 'indirect bandgap' material, and the actual onset of absorption is probably ~ 950 nm in this device. For electrolysis of HBr to H_2 and Br_2 , this corresponds to $U_{\text{loss}} \sim 0.7$ eV per photon. We mention this system to point out that rather low values of U_{loss} are possible in S4 systems. However, it is relatively easy to achieve low U_{loss} values in splitting HBr because a platinum electrode can produce Br_2 at a low overpotential. In contrast, to carry out reaction (1) it is necessary to generate O_2 , which is generally difficult without suffering a large charge-transfer overpotential.

A fourth example is the reaction of green-plant photosynthesis:



This reaction has $E^\circ = 1.24$ V, virtually the same as reaction (1), so the considerations of efficiency are the same. Although the plant uses two somewhat different photosystems, they have nearly the same $\lambda_g \approx 690$ nm ($U_g \approx 1.80$ eV) and are intermixed in the leaf. Two photons are used to drive each electron, so even though no H_2 is generated, this system is also equivalent to the S4 case analysed above. Each photon must supply 0.62 eV to drive the reaction; hence $U_{\text{loss}} = 1.18$ eV and, from equations (5)–(7), the maximum conversion efficiency would be 12.4% if all light with $\lambda \leq 690$ nm were absorbed and used to drive reaction (11) with $\phi_{\text{conv}} = 1.0$. When real measured *in vivo* quantum yields and absorption coefficients (for example, in a leaf) are used, the gross efficiency drops to $\sim 9\%$ (refs 10, 40). Note, however, that if the photosynthesis reaction were to be driven by an S2 scheme with $U_{\text{loss}} = 1.18$ eV per photon, λ_1 could not be $> \sim 510$ nm and the maximum efficiency would be only $\sim 9\%$.

As a final example, we note that several investigators have studied photoelectrochemical cells with a photoanode and a photocathode in series, using different semiconductors for the two photoelectrodes⁴¹⁻⁴³. For example, a cell with a photoanode of n-TiO₂ ($\lambda_g \approx 410$ nm) and a photocathode of p-GaP ($\lambda_g \approx 550$ nm) will split water, although the efficiencies achieved have been rather low. These experiments were performed with the electrodes side by side, but in principle the GaP electrode could be placed behind the TiO₂ electrode, and this would be an example of a D4 system. The two bandgaps are not well matched for equal absorbed photon fluxes, and $U_{\text{loss}} \approx 2.0$ eV in this example, so it serves mainly to show that the concept of such D4 systems is workable. However, there are practical difficulties with layered systems, such as providing transparent ohmic contacts.

Realizable efficiencies

Up to this point the only non-ideal factor we have considered is an increase in U_{loss} . We concluded that $U_{\text{loss}} \approx 0.8$ eV is a realistic goal for a practical system, which leads to calculated maximum efficiencies of $\sim 17\%$ ($\lambda_1 \approx 610$ nm) for S2 systems and $\sim 27\%$ ($\lambda_1 \approx 720$; $\lambda_2 \approx 1,120$ nm) for D4 systems. In practical devices, however, there are other losses to be considered: (1) Reflection losses. The collector in which the photochemical reaction occurs must have a transparent cover. Each surface through which the sunlight passes results in a reflection loss of ~ 4 – 5% for untreated glass at normal incidence and considerably more at higher angles of incidence⁴⁴. These losses can be reduced with anti-reflection coatings, but at added cost. We shall assume that total reflection losses can be kept to 10% . (2) Quantum-yield losses. We have already seen that it is difficult to achieve quantum yields (ϕ_{conv}) close to the optimum values for each scheme. We shall be optimistic and assume that quantum yields can be improved to 90% of the ideal values. (3) Absorption losses. In our calculations we have assumed that all sunlight with $\lambda \leq \lambda_g$ is absorbed and used in driving reaction (1). In real systems the absorption coefficient will vary with wavelength and some sunlight will be absorbed by inactive components. We feel that a realistic goal would be that $\sim 80\%$ of sunlight with $\lambda \leq \lambda_g$ can be absorbed and used. (4) Collection losses. The product gases (at least H_2) must be collected and probably compressed for

storage. There are significant inherent losses in these processes, but we shall be optimistic and assume a collection and storage efficiency of ~90%.

There may be other losses we have not considered; however, when the above estimates are taken into account with $U_{\text{loss}} \sim 0.8$ eV, S2 systems would have efficiencies of ~10%, whereas D4 systems could achieve overall efficiencies of ~16%. If 10% is taken to be a lower limit for a practical device, it is clear that D4 systems have the best chance of realizing such a goal. We have not included 'usage' losses involved in the transportation and use of hydrogen, because these are losses which will be present irrespective of the source.

In summary, claims of high efficiencies for solar water photo-

lysis must be viewed with caution. One must pay especially close attention to the mechanism(s) of the processes and the way in which the efficiencies are calculated³⁹.

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ARTICLES

Response of global deep-water circulation to Earth's climatic change 135,000–107,000 years ago

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The response of deep ocean circulation to major climatic events of the past 135,000 years is inferred using carbon-13 analyses of benthic foraminifera from the major ocean basins. The results demonstrate that the circulation of the world ocean deep water is very sensitive to climate and that it changes drastically during climatic transitions.

COLD bottom and deep waters are found in all ocean basins because, in very limited areas of the world ocean at high latitudes, low-temperature surface water becomes sufficiently dense to sink to a great depth. Since gases are exchanged only at the ocean surface, the deep circulation alone is responsible for the continuous supply of dissolved O_2 , permitting life in the deep sea. The deep water circulation may also play an important part in large-scale climatic fluctuations such that major changes in the deep water circulation are associated with Pleistocene glacial advances and retreats. At least three climatic models have been proposed that also take into account the deep circulation of the oceans¹⁻³. Although none of these models is entirely supported by palaeoclimatic evidence⁴⁻⁶, many deep-sea core studies have suggested that the deep water circulation was drastically different from the present one during the last ice age. For instance,

isotope evidence indicates that the Norwegian Sea was not a source of cold deep water during the last ice age as it is today⁷.

Until now, all studies have dealt with time series of a proxy-indicator in only a few locations. Such studies of deep water circulation used benthic foraminiferal species variation or chemical and isotope composition changes in benthic foraminiferal shells and suggested two major results: (1) that North Atlantic deep water (NADW) was generally forming during the last glaciation; and (2) that its dissolved O_2 content was low because its net production was substantially diminished⁸⁻¹⁰. In this paper, we use a more global approach. Duplessy *et al.*¹¹ demonstrated that the geographical distribution of the ^{13}C isotope composition of Holocene benthic foraminifera reflects that of the total CO_2 dissolved in oceanic deep water. The changes in the global deep water circulation are shown by

Table 1 Core location and depth and O and C isotope composition of benthic foraminifera at three core levels deposited during major climatic events of the period 135,000–107,000 years ago

Core	Lat.	Long	Depth (m)	Isotope stage 6			75% Deglaciation			Isotope substage 5d		
				Depth (cm)	δ ¹⁸ O	δ ¹³ C	Depth (cm)	δ ¹⁸ O	δ ¹³ C	Depth (cm)	δ ¹⁸ O	δ ¹³ C
CHN115-70*	29°55' N	35°39' W	2,340	140	+4.96	+0.29						
CHN115-89*	30°53' S	38°12' W	3,152	100	+4.83	−0.05						
CHN115-90*	30°51' S	38°22' W	3,384	106	+4.94	0.00						
CHN115-91*	30°50' S	38°26' W	3,576	80	+4.79	+0.27						
CH72-02	40°36' N	21°42' W	3,485	216	+5.14	+0.05	188	+3.73	+0.74	169	+4.25	+0.75
CH73-139C	54°38' N	16°21' W	2,209	740	+5.72	+0.51	710	+3.57	+0.43	600–610	+4.62	+0.60
D-114	41°45' N	51°56' W	3,732					+3.68	+0.41			
D-117	42°06' N	52°45' W	3,300	290	+4.87	+0.24						
DSDP 508	00°32' N	86°05' W	2,783	810	+4.91	−0.28	730	+3.65	−0.15			
DSDP 552A	56°03' N	23°13' W	2,311		+5.21	+0.61						
K-11	71°47' N	01°36' E	2,900							255	+5.00	+1.31
M12-392	25°10' N	16°50' W	2,573	883–963	+4.95	−0.62				553	+4.82	−0.01
M13-519†	05°40' N	19°51' W	2,862	203	+5.10	+0.01						
MD73-025	43°49' S	51°19' E	3,284	1,400	+5.08	−0.19	1,371	+3.77	+0.05			
MD73-026	44°59' S	53°17' E	3,429				1,640–1,650	+3.59	+0.10			
MD76-135	14°27' N	50°31' E	1,895							840	+3.90	−0.62
MD77-202	19°13' N	60°41' E	2,427	850	+5.19	−0.35	820	+3.58	−0.09	640	+4.14	−0.19
MD79-254	17°53' S	38°40' E	1,934	900	+5.17	+0.32	880	+3.90	+0.38	750	+4.22	+0.36
NO79-28	45°38' N	22°45' W	3,625	590	+5.53	−0.08				480	+4.42	+0.53
RC8-39	42°53' S	42°21' E	4,330	925–931	+5.04	−0.23						
RC10-65	00°41' N	108°37' W	3,588			−0.17			−0.19			
RC12-294	37°16' S	10°06' W	3,308	306–308	+5.02	−0.02	282	+3.52	+0.37			
RC12-339	09°08' N	90°02' E	3,010	348–350	+4.69	−0.22	334	+3.66	−0.52	300	+4.09	+0.14
RC13-205	02°17' S	05°11' E	3,731	246	+5.30	−0.57				198–200	+4.33	+0.32
RC13-228	22°20' S	11°12' E	3,204	675–680	+5.18	−0.24	635–640	+3.73	+0.21	585	+4.24	+0.05
RC13-229	25°30' S	11°18' E	4,191	330	+4.86	−0.62	294	+3.66	−0.02	280	+4.10	−0.16
SU81-29	41°35' N	10°57' W	3,068	550	+5.49	−0.26	502	+3.79	+0.20	420	+3.79	+0.56
SU81-32	42°06' N	9°47' W	2,280	730	+5.51	−0.04				540	+4.57	+0.92
SU81-39	45°22' N	10°32' W	2,620	90	+5.31	+0.33				60	+4.56	+0.84
V19-28	02°22' S	84°39' W	2,720			−0.17			−0.19			
V19-30	03°23' S	83°21' W	3,091	823–826	+5.28	−0.38	770–773	+3.56	−0.08	706–713	+4.37	−0.38
V21-146	37°41' N	163°02' E	3,968	400	+5.28	−0.25	375–378	+3.79	+0.04	340	+4.28	+0.28
V22-182	00°33' S	17°16' W	3,937	426	+5.04	+0.05				342	+4.33	+0.47
V22-196	13°50' N	18°58' W	3,728	667–682	+5.14	−0.36	587–592	+3.63	+0.15	502–507	+4.39	0.00
V23-82	52°35' N	21°56' W	3,974	840	+4.71	−0.10				683–696	+3.76	+0.48
V23-100	21°18' N	21°41' W	4,579	170–180		+0.22						
V25-59	01°22' N	39°29' W	3,824	380	+4.94	+0.06	355–357	+3.63	+0.52	322.5	+3.81	+0.86
V27-20	54°00' N	46°12' W	3,510	519	+4.70	+0.56	509–514	+3.60	+0.36	454	+4.09	+1.23
V27-60	72°11' N	08°34' E	2,525							485–500	+4.99	+1.22
V27-86	66°36' N	01°07' E	2,900							295	+5.24	+1.27
V28-14	64°47' N	29°34' W	1,855	545	+5.10	+0.93	535	+3.67	−0.23	501	+4.14	+1.20
V28-238	01°01' N	160°29' E	3,120	235	+4.84	0.00						
V28-304	28°32' N	134°08' E	2,942	480	+4.79	−0.08						
V28-345	17°40' S	117°57' E	1,904	596	+4.70	−0.06				516	+3.82	+0.67
V29-179	44°00' N	24°32' W	3,331	555	+5.37	−0.30				470–480	+4.31	+0.52
V30-97	41°00' N	32°56' W	3,371	640	+5.20	−0.50	582	+3.38	+0.53	538	+4.03	+0.50
V32-128	36°28' N	177°10' E	3,623	321	+4.84	−0.46						
V34-88	16°31' N	59°32' E	2,100	565	+4.49	−0.04	530	+3.61	+0.11	470	+3.73	+0.11
Y71-6-12	16°26' S	77°33' W	2,734	192	+5.08	+0.08	159	+3.77	−0.11			
Y72-11-1	43°15' N	126°23' W	3,000	1,260–1,312		−0.57						

$\delta^{18}\text{O}$ values normalized to *Uvigerina*; $\delta^{13}\text{C}$ values normalized to *Cibicides*. * From ref. 43, † from ref. 44.

comparing maps of the $^{13}\text{C}/^{12}\text{C}$ ratio of benthic foraminifera that lived in deep waters 135,000–107,000 yr ago. The penultimate glacial to interglacial transition and the interglacial to glacial transition which followed are characterized using the benthic foraminiferal isotope analyses that were made as a part of the last interglacial ocean study¹² and those from other measurement programmes. The core locations and isotope values are reported in Table 1.

Hydrological and geochemical basis

Sinking surface waters carry a concentration of dissolved O_2 almost in equilibrium with the atmosphere and a low total CO_2 content resulting mainly from carbon use by phytoplankton for photosynthesis¹³. After their formation, deep waters do not constitute a closed system and they are subject to the addition of CO_2 from two sources, the oxidation of organic matter,

constantly supplied from the surface^{14,15}, and the dissolution of the carbonate of skeletal parts of organisms that live in the overlying waters, but sink towards the bottom after death. Because the organic matter is strongly depleted in ^{13}C (refs 16,17), the CO_2 produced from the oxidation of organic matter has a $\delta^{13}\text{C}$ of -20 to -25 per mil. If this were the only source, the concentration of total CO_2 would be negatively correlated with $\delta^{13}\text{C}$ and with the water O_2 content along the flow path of a deep water mass. In contrast, the CO_2 produced from the dissolution of biogenic carbonate has a $\delta^{13}\text{C}$ fairly close to that of total CO_2 (ref. 18). Consequently, the $\delta^{13}\text{C}$ variations of total CO_2 in the world deep ocean will depend on the relative importance of these two local sources of total CO_2 and are closely correlated with those of the dissolved O_2 content (Fig. 1).

In the Norwegian Sea, surface samples have high O_2 content and high $\delta^{13}\text{C}$ values. The deep waters which are formed by

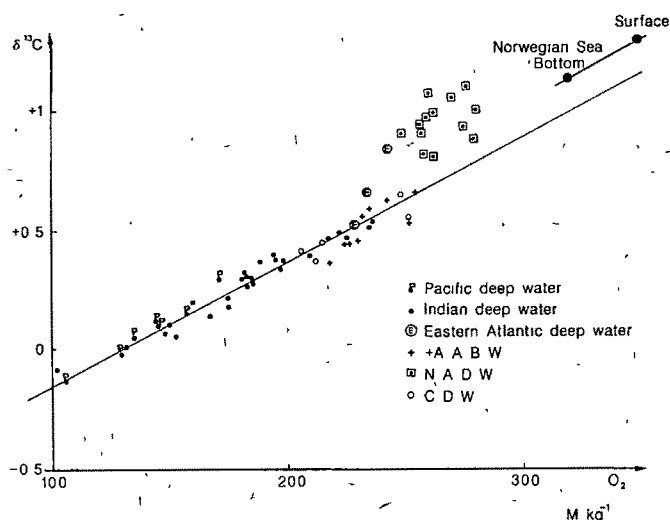


Fig. 1 $\delta^{13}\text{C}$ in total dissolved CO_2 plotted against dissolved O_2 . Selected data from the deep water masses taken from GEOSECS files and from ref. 45. Pacific values (P) have been measured in deep waters (below 300 m) from stations SCAN X 20, 30, 38, 43 and 56 (ref. 45) and GEOSECS 323 and 347. Indian deep waters (●) (below 3,000 m) data from GEOSECS stations 413, 416, 417, 418, 420, 421, 424, 425, 427, 428, 429, 430, 431, 433, 435, 436, 438, 441, 444, 445, 446, 447, 448, 450, 452 and 453. AABW values (⊕) from GEOSECS stations 33, 39, 42, 46, 49, 53, 55, 64, 68 and 72 below 4,000 m. Eastern Atlantic deep waters (+) from GEOSECS stations 103, 107 and 113 below 3,000 m. Circumpolar current data (○) from GEOSECS stations 76, 78, 82, 89 and 91 below 3,000 m. NADW data (□) from the core of this water mass, as recognized by Kroopnick (refs 20–21), at GEOSECS stations 1, 3, 5, 24, 29, 30, 31, 33, 36, 39, 42, 46 and 49. Each point is the mean of the values measured in the depth range in which the dissolved O_2 is constant; this procedure reduces the scatter in the raw data.

the cooling and sinking of surface waters are the most enriched in ^{13}C in the world ocean. As a result, in the western North Atlantic Ocean, within the core of the southward-flowing NADW¹⁹, the $\delta^{13}\text{C}$ of total CO_2 is high and does not vary significantly, because the North Atlantic is an area of relatively low surface productivity^{20,21}.

In the South Atlantic Ocean, lower $\delta^{13}\text{C}$ values are found as a consequence of the northward flow of Antarctic bottom water (AABW). Dense bottom waters are formed around the Antarctic continent, in the Weddell and the Ross Sea²², without exchanging a noticeable amount of gas with the atmosphere. These bottom waters strongly mix with the overlying Circumpolar water (CPW) and the resulting water masses, which extend northward into each of the deep ocean basins, all have a low dissolved O_2 content and a low $\delta^{13}\text{C}$ value.

The AABW found in the western Atlantic Ocean northward to 30° N can be identified by its low $\delta^{13}\text{C}$ value²¹. Progressive mixing with NADW results in both an O_2 and a $\delta^{13}\text{C}$ increase along this northward-flowing water mass. In the Indian and Pacific Oceans, the major process occurring in the deep water is the *in situ* consumption of organic matter. As a consequence, bottom O_2 concentration and total CO_2 $\delta^{13}\text{C}$ values decrease northwards. A linear relationship is observed between $\delta^{13}\text{C}$ and dissolved O_2 (Fig. 1), indicating that at least in this water mass, $\delta^{13}\text{C}$ is a good proxy-indicator for dissolved O_2 content. The same relationship also applies to AABW, reflecting the common origin of these various water masses.

In the eastern Atlantic Ocean, bottom waters from the south penetrate the Cape Basin but are blocked by the Walvis Ridge. The O_2 content and $\delta^{13}\text{C}$ value of the water below 3,000 m in the Cape Basin are not different from that of AABW and fall on the straight line characterizing waters originating from the south. The more northern basins are filled by bottom water which flows from the western basin of the Atlantic through the Romanche and Vema Fracture Zones in equatorial latitudes.

These waters progressively mix with North Atlantic and Mediterranean waters that are richer in ^{13}C , and their characteristics are intermediate between NADW and AABW.

In summary, the modern distribution of the $\delta^{13}\text{C}$ value of the total CO_2 dissolved in deep and bottom waters of the ocean shows that total CO_2 $\delta^{13}\text{C}$ can be used as a tracer of the deep water circulation under the following conditions: (1) within a deep water mass, the $\delta^{13}\text{C}$ value decreases with increasing oxidation of organic matter, such that the $\delta^{13}\text{C}$ decrease is related both to the surface productivity and to the time elapsed since the deep water was isolated from the atmosphere; (2) when two water masses are compared, the $\delta^{13}\text{C}$ value in each depends not only on their residence time at depth, but also on the ratio between the CO_2 produced from organic matter oxidation and that derived from carbonate dissolution; and (3) mixing between two water masses results in $\delta^{13}\text{C}$ values intermediate between the two original components, regardless of chemical reactions occurring at depth.

^{13}C record of benthic foraminifera

Changing oceanographic conditions that result in changes in both dissolved O_2 content and $\delta^{13}\text{C}$ of total CO_2 , may be reconstructed from the down core ^{13}C record in a deep-sea core. One additional factor which must be taken into account is temporal changes in the $\delta^{13}\text{C}$ content of the global ocean reservoir, which would result from changes in the global biomass^{23,24}.

The $\delta^{13}\text{C}$ value of *Cibicidoides wuellerstorfi* and *C. kullenbergi*, which differs from that of total CO_2 by only $+0.07 \pm 0.04$ per mil¹¹, has been used as a good estimate of the C isotope composition of total CO_2 in the past. This $\delta^{13}\text{C}$ value is 0.90 permil heavier than that of *Uvigerina* at the 95% confidence level. Other species have also been calibrated by comparison with *Uvigerina* or *Cibicidoides*²⁵. Therefore, even if a single benthic foraminiferal species is too rare for analysis throughout the length of a deep-sea core, a complete record of the variations of total CO_2 $\delta^{13}\text{C}$ can be obtained from analysis of several taxa after adjustments have been made to compensate for isotope departure of the taxa from the reference species (*C. wuellerstorfi*).

Figure 2 compares four C and O isotope records of benthic foraminifera in deep-sea cores raised from various ocean basins for the period 135,000–107,000 yr BP. The oxygen isotope records are very similar, in agreement with the hypothesis that the major signal in these records originates from the waxing and waning of large volume of isotopically light water in the Northern Hemisphere ice sheets during the major part of the Pleistocene^{26–29}.

By contrast, the C isotope records exhibit noticeable differences: the amplitude of $\delta^{13}\text{C}$ variations is much larger in the Atlantic cores than in the Indian and Pacific cores⁷. During the transition between glacial stage 6 and interglacial stage 5, $\delta^{13}\text{C}$ values increase in the four cores, but the $\delta^{13}\text{C}$ record lags behind the $\delta^{18}\text{O}$ record in the Atlantic while both records are in phase in the Pacific and Indian Oceans.

Because the $\delta^{13}\text{C}$ value of benthic foraminifera reflects that of the total CO_2 dissolved in the oceanic deep water, the observed differences between the various $\delta^{13}\text{C}$ records indicate that the $\delta^{13}\text{C}$ of total CO_2 in the past ocean has changed not only because of changes in the amount of carbon stored in the exchangeable reservoirs (atmosphere, biosphere and ocean), but also because of changes in the deep water circulation and in the oceanic carbon chemistry^{23,24,30}. Consequently, the major trends of deep water circulation and chemistry in the world ocean during climatic conditions different from those of today can be reconstructed by comparing maps of the $\delta^{13}\text{C}$ of benthic foraminifera that lived at specified times in the past.

Glacial to interglacial contrasts

The $\delta^{13}\text{C}$ distribution of the total CO_2 dissolved in the deep ocean during the last interglaciation (isotope stage 5e, ~122,000 yr ago) has been reconstructed (Fig. 3a) from $\delta^{13}\text{C}$ values of benthic foraminiferal calcite in 41 deep-sea cores raised

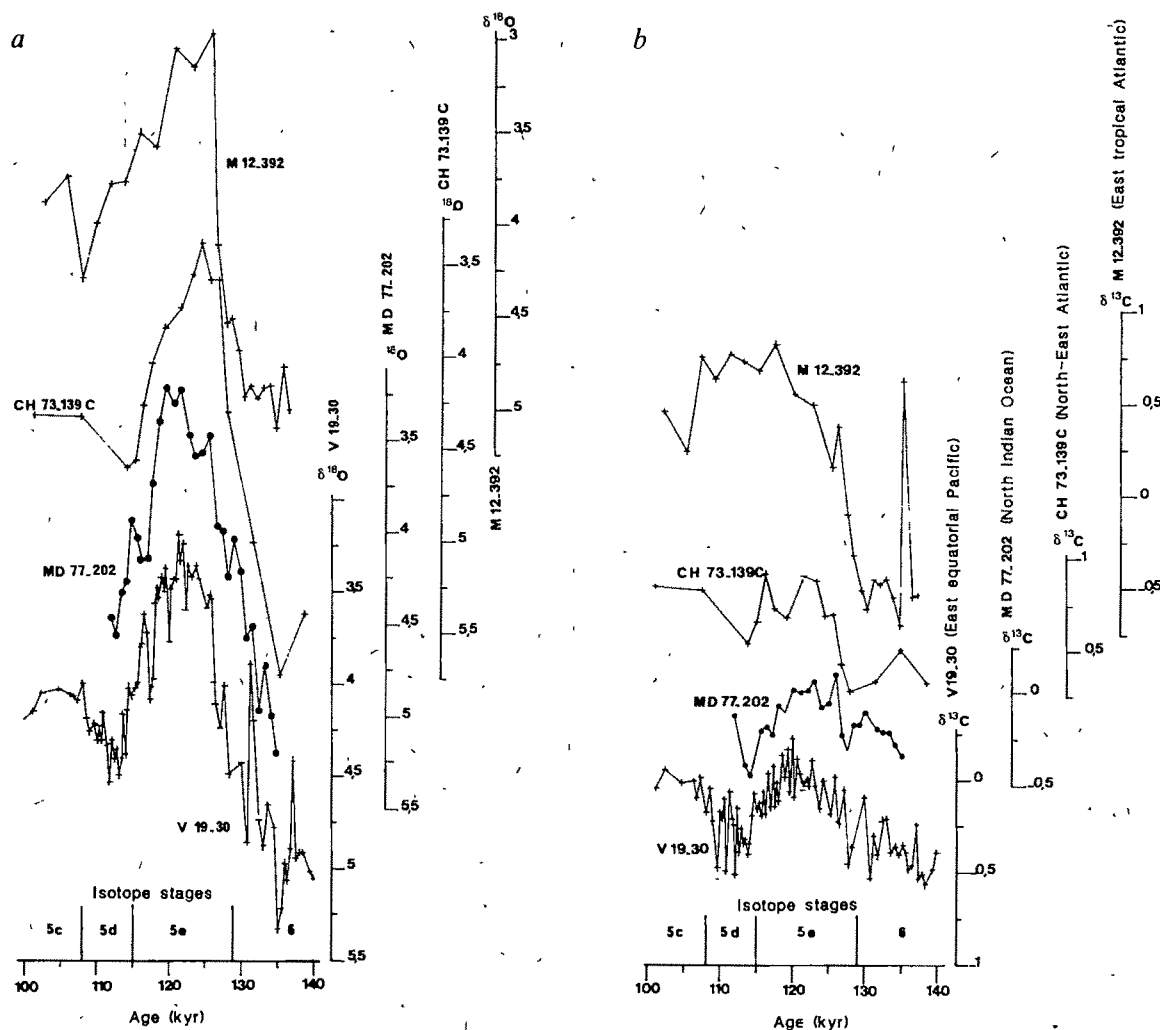


Fig. 2 Down core ^{18}O (a) and ^{13}C (b) record from 135 to 107 kyr in four cores, illustrating the temporal change in ^{13}C in distinct regions over the interval covered by this study. The timescale used for the plots is the SPECMAP timescale³³. Core CH 73.139C comes from Rockall Plateau, core M12-392 has been raised from the continental margin off Africa in the tropical eastern Atlantic Ocean. Cores MD 77-202 and V19-30 come respectively from the northern Indian Ocean and from the Panama Basin in the eastern equatorial Pacific, where deep waters are today much older and much more depleted in dissolved O_2 and ^{13}C than those the Atlantic.

from the major ocean basins (see Table 3 of ref. 11 for a list of measured isotope values). This reconstruction shows that deep water sources were active both in the Northern and Southern Hemispheres, both because the highest isotope values are found in the Norwegian Sea and because the $\delta^{13}\text{C}$ values decrease in the Indian and Pacific deep waters according to a pattern similar to present-day conditions. However, the production of AABW was stronger than that of NADW, resulting in an east-west $\delta^{13}\text{C}$ gradient in the Atlantic Ocean higher than the modern one¹¹.

The $\delta^{13}\text{C}$ value of the benthic foraminiferal calcite for each core location during the preceding glacial maximum (isotope stage 6 maximum, ~135,000 yr ago) is reported (Fig. 3b) and the glacial anomaly ($\delta^{13}\text{C}$ difference between the glacial value and the interglacial value) shown in Fig. 3c.

No benthic foraminifera have been found in Norwegian Sea cores V28-60 and K-11 although stage 6 is well represented in the planktonic record. We interpret the absence of benthic organisms as an indication that the Norwegian Sea deep water was sufficiently depleted in O_2 and nutrients to reduce drastically the benthic productivity. This implies no renewal of the deep water and hence that no deep water formed in this basin. The highest $\delta^{13}\text{C}$ values and the smaller anomalies are found in the North Atlantic Ocean (Fig. 3b, c), which indicates the formation of deep water in the northernmost part of this basin, close to the limit of permanent sea-ice³¹. However, in the western Atlantic, the southward extension of this water mass at a depth deeper

than 3,000 m was limited to the high latitudes (core V27-20), because low $\delta^{13}\text{C}$ values are found south of 50°N . By contrast with the interglacial pattern, the western Atlantic was therefore mostly filled by water low in dissolved O_2 and ^{13}C , most probably originating from the Antarctic Ocean, like AABW today.

The lowest $\delta^{13}\text{C}$ values (Fig. 3b) and the highest anomalies ($\delta^{13}\text{C}$ difference between isotope stage 6 and 5e; Fig. 3c) are found in the eastern Atlantic Ocean. This is observed primarily along the coast of Africa. The low $\delta^{13}\text{C}$ values contrast sharply with the interglacial pattern (Fig. 3a) and indicate the production of large amounts of ^{13}C -depleted CO_2 resulting from the oxidation of organic matter produced by the enhanced upwelling activity in this area³². As a consequence, very low $\delta^{13}\text{C}$ values are found off the western coast of Africa (M12-392) and of Portugal (SU81-32). This phenomenon, however, is not strictly coastal and it can be inferred that surface productivity was also enhanced far from the coast, because low $\delta^{13}\text{C}$ values are found in the central North Atlantic Ocean, north of 40°N and below 3,000 m. The $\delta^{13}\text{C}$ values were plotted against depth in the 12 cores north of 20°N for stage 6 and 5e (Fig. 4a, b). The resulting $\delta^{13}\text{C}$ profiles show very distinct features. The interglacial profile exhibits constant $\delta^{13}\text{C}$ values, reflecting an hydrological pattern similar to that of today. On the other hand the glacial profile indicates a strong $\delta^{13}\text{C}$ minimum around 3,200 m and the occurrence of two very different water masses. The first mass was shallower than 2,500 m and consisted of O_2 -rich, high- $\delta^{13}\text{C}$

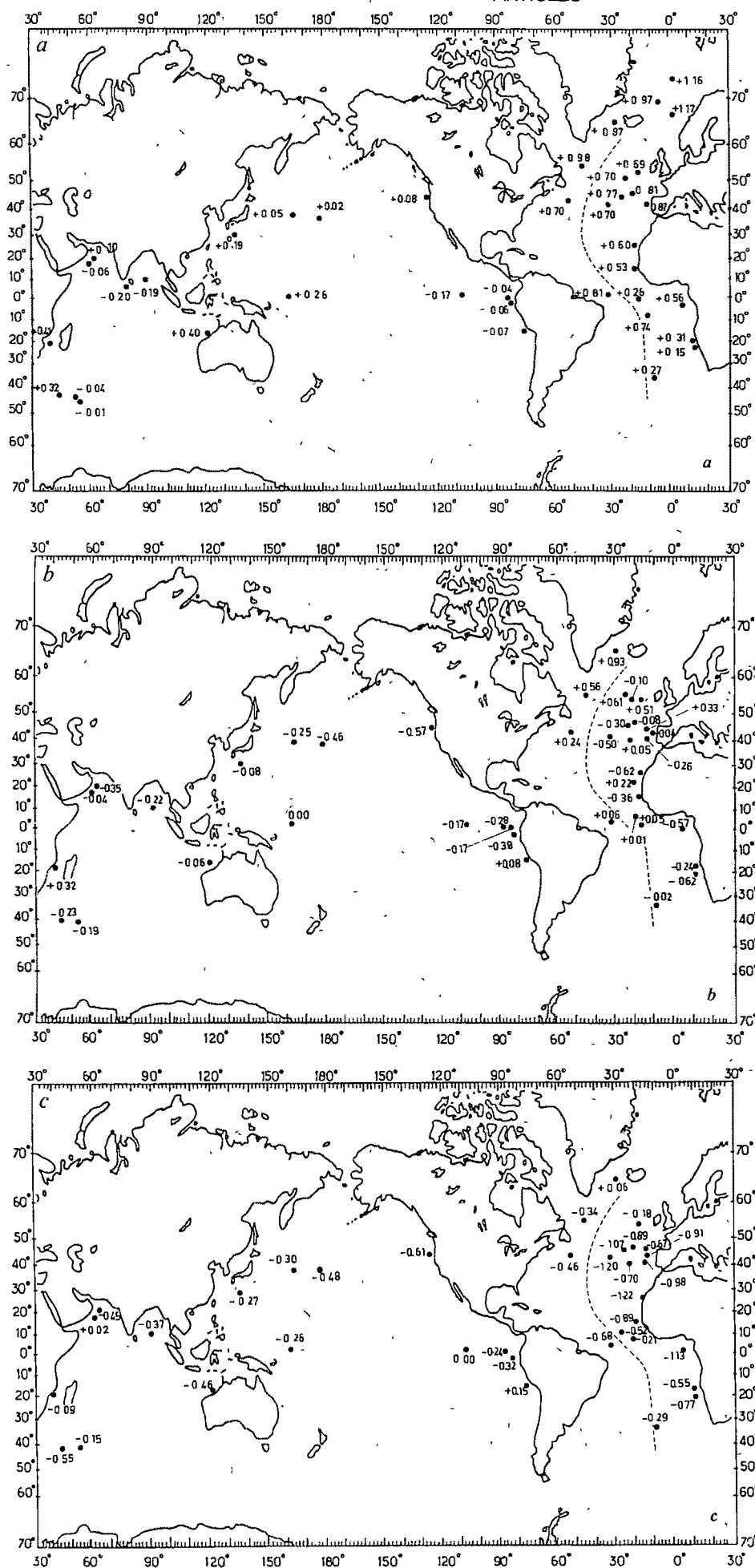


Fig. 3 Estimates of $\delta^{13}\text{C}$ values of total CO_2 in the oceanic deep water for the last interglaciation (a) and for the penultimate glaciation (b). The glacial anomaly (c) is calculated as the $\delta^{13}\text{C}$ difference between glacial and interglacial values at the same core site. Dashed line indicates the position of the crest of the Mid-Atlantic Ridge.

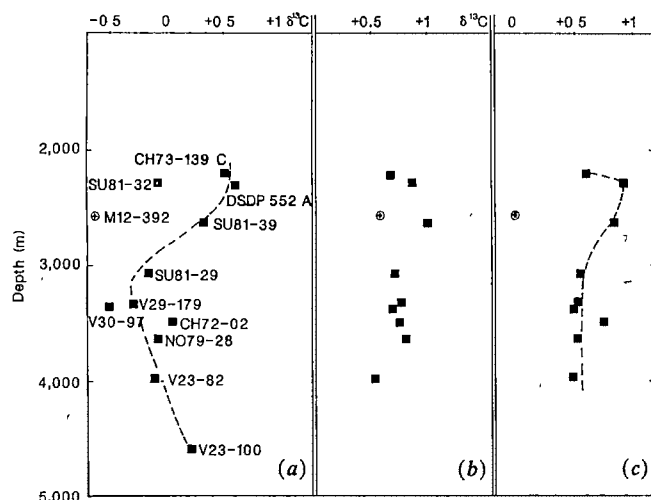


Fig. 4 Depth profiles of $\delta^{13}\text{C}$ estimates for total CO_2 in deep waters from the Eastern Atlantic Ocean north of 20°N for (a), the penultimate glaciation (isotope stage 6); (b), the last interglaciation (isotope substage 5e); and (c), the beginning of the glaciation (isotope substage 5d).

water which originated in the North Atlantic. The second was a deeper water mass, which was much more depleted in dissolved O_2 and ^{13}C , and probably originated from the western Atlantic deep water. It crossed the Mid-Atlantic ridge through the equatorial fracture zones in the same manner as today. Within this water mass, strong *in situ* consumption of O_2 resulted in a well-developed $\delta^{13}\text{C}$ minimum. Mixing between this water mass and glacial NADW resulted in sharp $\delta^{13}\text{C}$ change with depth in the north-eastern Atlantic Ocean (Fig. 4). This feature is one of the most dramatic aspects of circulation changes between glacial and interglacial periods. Another area exhibiting a strong $\delta^{13}\text{C}$ gradient was the north-western Atlantic Ocean, which was the zone of mixing of the southwards-flowing glacial NADW with the northwards-flowing AABW.

In the Indian and Pacific Oceans, the magnitude of the $\delta^{13}\text{C}$ anomalies is smaller than that of the Atlantic Ocean, suggesting that the stage 6 pattern of deep water circulation was similar to the interglacial one. No indication of formation of deep water and sinking below 1,500 m is provided by the cores raised from the North Pacific Ocean, but our coverage is rather poor and

additional cores are necessary to investigate the sinking of surface water at intermediate depth in the North Pacific. The stronger $\delta^{13}\text{C}$ anomalies are found in the northern and north-eastern area (cores V32-128 and Y72-11-1). The $\delta^{13}\text{C}$ gradient between the equatorial and north-east Pacific is stronger than the modern one, suggesting that the residence time of the Pacific deep water was greater than that of today. A weaker deep-water circulation is also supported by the large anomaly measured in cores raised from the Circumpolar ocean (RC8-79; MD73-025).

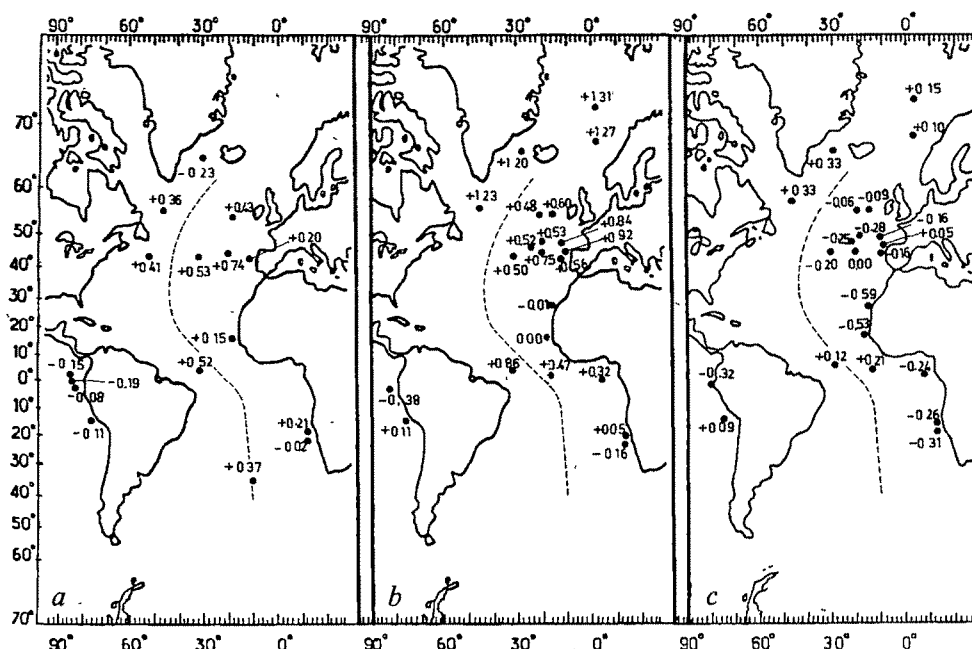
Therefore, the pattern of deep water circulation for glacial isotope stage 6 is characterized by weaker fluxes than those of interglacial time and an important reduction in the amount of deep water formed in the Northern Hemisphere, so that most oceanic basins were filled with water originating from the Southern Ocean.

Disappearance of NADW

During the transition from glacial isotope stage 6 to interglacial isotope stage 5e, $\delta^{18}\text{O}$ values of benthic calcite changed from $\sim +5$ per mil to $+3.1$ per mil. To understand the impact of the deglaciation on the deep water circulation, we selected in the cores the level characterized by a $\delta^{18}\text{O}$ value close to $+3.6$ per mil, which would correspond to $\sim 75\%$ of total deglaciation. Using the SPECMAP timescale³³, we estimated the age of this event at $\sim 127,000$ yr BP. In many cores, we have not found benthic foraminifera from that time, despite the detailed sampling made for the CLIMAP reconstruction¹². We believe that this time was unfavourable for benthic productivity, because of either poor oxygenation of the bottom or reduced input of nutrients if the surface productivity was low, as suggested by Ruddiman and McIntyre for the North Atlantic³⁴.

The $\delta^{13}\text{C}$ value of benthic calcite is reported in Fig. 5a. During the deglaciation, the northernmost core (V28-14) exhibits a very low $\delta^{13}\text{C}$ value. This value is much lower than both the glacial and the interglacial values and cannot be explained by any error resulting from bioturbation or foraminiferal abundance offset. Therefore, it indicates a very low renewal of the deep water, as expected in response to the stratification caused by the southward displacement of the limit of sea-ice³⁴. Further south, the $\delta^{13}\text{C}$ contrast between the northern and southern Atlantic Ocean had disappeared. The eastern Atlantic Ocean no longer showed isotopically low $\delta^{13}\text{C}$ values, indicating a sharp drop in the productivity of this basin. A comparison of this with the pattern for isotope stage 6 shows that the most dramatic $\delta^{13}\text{C}$ change during deglaciation occurred in those areas where well-oxygenated water was forming under glacial conditions (cores V28-

Fig. 5 Estimates of $\delta^{13}\text{C}$ values of total CO_2 in the oceanic deep water for (a), 75% of the deglaciation (127 kyr ago) and (b), for isotope substage 5d. The 5d anomaly (c) is calculated as the $\delta^{13}\text{C}$ difference between 5d and interglacial values at the same core site. Dashed line indicates the position of the crest of the Mid-Atlantic Ridge.



14, V27-20, CH73-139C). We interpret this as an indication that deep water formed neither in the northern Atlantic Ocean 127,000 yr ago nor probably during the entire interval of continental ice-melting.

In the Indian and Pacific Oceans, benthic foraminifera that lived during the deglaciation are also scarce. However, the measured $\delta^{13}\text{C}$ values for the benthic calcite are generally isotopically heavier than those of stage 6. As a $\delta^{13}\text{C}$ increase in the Pacific and Indian deep waters cannot occur without deep water circulation, our results demonstrate that some deep water formed in the Southern Ocean during the glacial to interglacial transition, when the North Atlantic deep water source had disappeared.

Enhanced NADW formation

During the phase of rapid ice growth which followed the last interglaciation, the eastern subpolar North Atlantic Ocean maintained warm sea surface temperatures comparable to those of the present-day ocean⁵. This pattern constitutes an optimal configuration for forming deep water in the northern Atlantic Ocean and in the Norwegian Sea. The ocean surface finally cooled late in isotope substage 5d, several thousand years after the mid-point of the ice-growth phase. We chose to reconstruct the $\delta^{13}\text{C}$ pattern of total CO_2 during the maximum of isotope substage 5d (ref. 33), ~107,000 yr ago, because this level can be accurately determined in the cores. At that time, a volume of ice that reached ~50% of the stage 2 full-glacial maximum formed over Northern America and Northern Europe^{35,36}. But we assume that the ocean deep water circulation remained similar to that which prevailed during the inception of the glaciation, because the $\delta^{13}\text{C}$ records do not exhibit major changes between the end of isotope substage 5e and the maximum of 5d (Fig. 2).

The $\delta^{13}\text{C}$ values of benthic calcite during isotope substage 5d are plotted in Fig. 5b and the 5d anomaly ($\delta^{13}\text{C}$ difference between substage 5d and 5e) in Fig. 5c. Isotopically heavy $\delta^{13}\text{C}$ values are found in the Norwegian Sea and in the north-western Atlantic Ocean. A strong positive anomaly in these basins indicate an enhanced flux of the Norwegian Sea overflow water into the North Atlantic Ocean. The high production of NADW may be related to the waxing of continental ice sheets that resulted in an increase of the world ocean salinity. This salinity increase was first experienced in surface waters, which must have received less runoff water during a period of strong evaporation⁵. This process increased the surface density and reduced the density difference between surface and bottom waters, and was therefore favourable to deep-water formation in high northern latitudes.

A western boundary current stronger than today prevailed in the North Atlantic Ocean and maintained a $\delta^{13}\text{C}$ difference between the eastern and western basins much larger than the modern one. Low $\delta^{13}\text{C}$ values and large negative anomalies along the coast of Africa indicate that upwelling was more active than during full interglacial conditions. In the Eastern Atlantic, the surface productivity and the *in situ* consumption of organic matter at depth were higher than today, resulting in the large $\delta^{13}\text{C}$ anomalies shown in Fig. 5c. In contrast with the pattern of stage 6, however, constant $\delta^{13}\text{C}$ values were observed between 2,200 m and 4,000 m, and no strong $\delta^{13}\text{C}$ minimum developed far from the coast (Fig. 4c).

Cores SU81-29 and SU81-32 exhibit a positive $\delta^{13}\text{C}$ anomaly (Fig. 4c). Both are on the path of the Mediterranean Sea overflow water. The Mediterranean Sea is the most important basin of the world ocean where evaporation produces a concentration effect on salt. The hydrological conditions are characterized by high salinity, low productivity and a residence time of the deep water shorter than one century. Consequently, $\delta^{13}\text{C}$ values of total CO_2 are high and similar to those of the Norwegian Sea³⁷. Today, the Mediterranean overflow water serves to increase both the $\delta^{13}\text{C}$ value^{21,37} and the salinity of the North Atlantic Ocean¹⁹. The high $\delta^{13}\text{C}$ values measured in cores SU81-29 and SU81-32 during isotope substage 5d can therefore be interpreted as an indication of a strong outflow of Mediterranean water into the

Atlantic Ocean. Such a pattern, which provides the North Atlantic drift with the high salinity required to form deep waters at high latitudes³⁸, would also serve to increase the production of NADW.

Our coverage is sparse in the Pacific and Indian Oceans and no distinct trends can be recognized, with the exception of low $\delta^{13}\text{C}$ values in the northern Indian Ocean. Because a noticeable decrease in the south-west monsoon was observed at that time³⁹, the upwelling activity was therefore lower than that of the preceding interglacial and the low $\delta^{13}\text{C}$ value of the deep water in the Northern Indian Ocean is best explained by a weaker renewal in this basin.

Wider implications

The reconstruction of the past deep water circulation, which has been a challenge during the past 10 yr, has now become a reality and our approach can be applied to other climatic events. The importance of the deep water circulation changes also suggests that these phenomena can be used to explain the variability of the carbon cycle and the decrease of the atmospheric CO_2 concentration during the last glaciation⁴⁰⁻⁴².

Our results demonstrate that the circulation of the world ocean deep water is very sensitive to climate change. The Southern Hemisphere source of bottom water seems to be one of the most constant features of the circulation of the deep waters. By contrast, the northern source of deep water exhibited a much greater variability, both in location and in intensity. This source was in the open North Atlantic Ocean during the penultimate glacial period and vanished during the following episode of continental ice melting that released a large amount of low salinity water and caused a strong stratification in the North Atlantic Ocean. This source reappeared in the Norwegian Sea during the last interglaciation, and its activity sharply increased during the inception of the last glaciation in response to the increasing salinity of the world ocean. Thus, we conclude that deep water circulation of the world oceans changes drastically during climatic transitions and constitutes an important part of the changing climate system.

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Palaeotectonic implications of increased late Eocene-early Oligocene volcanism from South Pacific DSDP sites

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Late Eocene-early Oligocene (42-35 Myr) sediments cored at two DSDP sites in the south-west Pacific contain evidence of a pronounced increase in local volcanic activity, particularly in close association with the Eocene-Oligocene boundary. This pulse of volcanism is coeval with that in New Zealand and resulted from the development of an Indo-Australian/Pacific Plate boundary through the region during the late Eocene. The late Eocene/earliest Oligocene was marked by widespread volcanism and tectonism throughout the Pacific and elsewhere, and by one of the most important episodes of Cenozoic climatic cooling.

Two continuous late Eocene through early Oligocene sedimentary sections were recovered during Leg 90 of the Deep Sea Drilling Project (DSDP). These are Sites 592 (36°28.40' S, 165°26.53' E; 1098 m water depth) and 593 (40°30.47' S; 167°40.37' E; 1,068 m water depth), both located in temperate latitudes to the west of New Zealand, on the southern Lord Howe Rise and the Challenger Plateau, respectively (Fig 1). Numerous layers of altered volcanic ash occur throughout the sequences and a 15-m unit of latest Eocene volcanoclastic sediments occurs in Site 593. The Eocene/Oligocene boundary is clearly marked by faunal and floral extinctions and by the universal, marine oxygen isotope shift in both sections. Previously, Site 277, located in the Subantarctic region south of New Zealand, was the only continuous oceanic section spanning the Eocene/Oligocene boundary in the South Pacific. All other sites containing Palaeogene sequences in this region exhibit

hiatuses associated with the Eocene/Oligocene boundary sediments^{1,2}.

Here we attempt to determine the age of the volcanogenic sediments in relation to established stratigraphical markers and discuss the implications of these late Eocene volcanogenic sediments with respect to the palaeotectonic development of both the south-west and the entire Pacific region.

Palaeoenvironmental and biotic events

The record of volcanic activity of late Eocene/earliest Oligocene age is significant because it coincides with a time of major palaeoceanographic change in the world's oceans³⁻⁵. Sequential changes in various biotic elements and in the oxygen isotope composition of calcareous microfossils mark the transition from relatively warm conditions of the middle Eocene to the cooler of the Oligocene. Oxygen isotope evidence indicates that the

cold waters of the oceans (psychrosphere) were generated during the late Eocene/earliest Oligocene when a glacial threshold was crossed in the Antarctic⁶⁻⁸. The record of deep-water cooling is shown first by an oxygen isotope ($\delta^{18}\text{O}$) enrichment at the middle/late Eocene boundary⁶. This deep-water cooling is also reflected by major changes in the deep-sea ostracod faunas between 43 and 40 Myr (ref. 7). Various planktonic faunal and floral elements exhibit a clearly defined trend towards decreasing diversity values during the late Eocene^{9,10}: calcareous nannoplankton exhibit a distinct (70%) reduction in diversity over a 7-Myr period in a stepwise fashion with the most abrupt reduction between the late middle and early late Eocene (41–40 Myr) and a further important reduction across the Eocene/Oligocene boundary¹⁰; planktonic foraminiferal assemblages also changed at several discrete intervals during the late Eocene, with the Eocene/Oligocene boundary marked by an increasing abundance of cool-water forms¹¹. These faunal and floral changes indicate the replacement of warm middle Eocene assemblages with successively cooler assemblages¹⁰⁻¹². The late Eocene cooling trend continued until the Eocene/Oligocene boundary⁵, where further marked cooling is represented by a rapid and distinct $\delta^{18}\text{O}$ enrichment of about 1‰ (refs 6, 13–15). This shift, isochronous according to planktonic foraminiferal biostratigraphy¹⁶, gave rise to maximum earliest Oligocene $\delta^{18}\text{O}$ values, which were followed by lower (lighter) values later in the Oligocene¹⁶. Associated with this event was a significant drop of up to 2,000 m of the calcium carbonate compensation depth and related oceanwide lithofacies changes¹⁷. Faunal and floral assemblages underwent distinct, but not drastic, catastrophic changes at the Eocene/Oligocene boundary^{12,18}.

The Eocene/Oligocene boundary is biostratigraphically linked with the last abundant disappearances of the *Globorotalia cerroazulensis-coccolensis* group, *Hantkenina* and *Globigerinatheka* and the rosette-shaped discoasters including *Discoaster barbadensis* and *D. saipanensis*¹⁹. These occur within a relatively brief interval between 36.1 and 37.4 Myr (base anomaly 13-top anomaly 15). An age of between 36.5 and 37 Myr has been suggested for the Eocene/Oligocene boundary¹⁹, which is close to that determined elsewhere (37.1–37.2 Myr)^{20,21}.

Deep-sea sequences

Site 592 provides a particularly fine, highly expanded 90-m section of late Eocene to early Oligocene sediment (Fig. 2). A distinct lithological change from calcareous ooze to chalk occurs below the extinction level of the foraminifer *Globigerinatheka index*. This extinction is often used to mark the Eocene/Oligocene boundary in south-temperate latitudes and is the marker used here. The underlying late Eocene sequence is largely represented by nannofossil chalk containing siliceous microfossils such as Radiolaria and sponge spicules, whereas the Oligocene is represented by surprisingly soft nannofossil ooze lacking biosiliceous material.

The extinction of *G. index*, marking the Eocene/Oligocene boundary, is in Core 36-3 between 90 and 64 cm. According to the calcareous nannoplankton markers, the Eocene/Oligocene boundary would be placed at the extinctions of *D. saipanensis* and *D. barbadensis*, which is in Core 37-2, between 5–6 and 33–34 cm. The time period between these two sets of extinctions is ~0.4 Myr (ref. 22). A similar sequence of extinctions of the two groups was recorded at DSDP Site 277 drilled south of New Zealand²³. Relevant species ranges are shown in Fig. 2, with assigned calcareous nannoplankton and planktonic foraminiferal zones.

The $\delta^{18}\text{O}$ record in Site 592 (Fig. 2) exhibits relatively constant late Eocene values followed by a rapid decrease (~0.4‰) and then a distinct increase (~1.0‰) beginning at the base of Zone NP21, which is considered to represent the Eocene/Oligocene boundary based on calcareous nannoplankton biostratigraphy. This oxygen isotope shift continued until the latest part of Zone NP21. The Eocene/Oligocene boundary based on planktonic foraminifera (last appearance of *G. index*) occurs near the

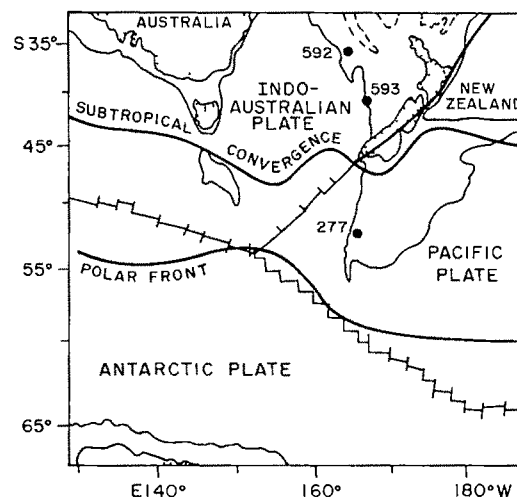


Fig. 1 Location of DSDP Sites 592, 593 and 277 in the south-west Pacific. Present-day positions shown of three lithospheric plates and their boundaries. Also shown are present-day positions of the Polar Front that separates Antarctic from Subantarctic surface waters and the Subtropical convergence that separates Subantarctic from cool-subtropical (temperate) water masses.

beginning of this oxygen isotope shift. The values then slightly decrease (~0.2‰) from the peak associated with the NP21/NP22 boundary and steadily decrease with little variation during the remaining early Oligocene. There is remarkable parallelism of planktonic and benthic oxygen isotope variations. Detailed interpretations of the oxygen isotope records for Sites 592 and 593 are provided elsewhere²⁴.

Site 592 contains many (4–29 laminae per 9 m core length) thin, altered, pale green volcanic ash layers (Fig. 2) in the late Eocene to early Oligocene (~42–35 Myr). Such layers are absent in the Neogene section of Site 592 except for rare occurrences in the middle Miocene and common occurrences (2–12 laminae per 9 m core length) in the Quaternary^{25,26}.

A spectacular sequence over the Eocene/Oligocene boundary is recorded at Site 593 (Fig. 3). Close to the boundary is a remarkable 15-m sequence of greyish black to greyish green, well-lithified volcanoclastic breccia, sandstone and mudstone, including possible ash tuff, between 546 and 561 m. These sediments are basaltic in composition and are interpreted as proximal turbidites, debris flows and pyroclastic flows²⁷. They represent the 'only non-carbonate lithological units drilled at either of the two sites, and are rare for open-ocean pelagic sequences. The unit consists of at least 40 subunits that exhibit finer-grained sediments upwards, and are, typically 20–50 cm thick. Several of the subunits exhibit a partial Bouma sequence of sedimentary structures (dominantly divisions A and B). The internal structure of the individual subunits, especially the predominance of Bouma A and B divisions compared with B and E divisions, suggest proximal rather than distal deposits, although some of the coarser beds may be submarine pyroclastic flows. The source is almost certainly the nearby buried Lalitha Volcanic Pinnacle^{25,27}, ~2 km from Site 593, and conspicuous on seismic records. The volcanoclastic unit can be traced for several tens of miles to the north on the seismic profile. This unit is confined to a relatively short time interval because most of the remaining seismic section is marked by relatively acoustic transparency²⁵. The volcanoclastic rocks are underlain by nanno chalk at the base of the hole that contains many thin laminae of altered volcanic glass indicating episodes of explosive volcanism. As in Site 592, the Eocene/Oligocene boundary, as defined by the extinction of *G. index*, is again younger than that marked by the extinction of *D. saipanensis* (Fig. 3). The volcanoclastic unit was emplaced between these two dates and is thus of latest Eocene age. The Eocene/Oligocene boundary occurs at ~3.38 m above the top of the volcanic unit at ~57–90 cm (Fig. 3). The extinction of *D. saipanensis* occurs at the base of the volcanic

Fig. 2 Late Eocene early Oligocene biostratigraphy and oxygen isotopic stratigraphy of DSDP Site 592 showing quantitative distribution of altered volcanic ash laminae in the sequence. Recovered sediment sections shown by black in left column. Oxygen isotopic curves are for the benthic foraminifer *Oridorsalis umbonatus* and the planktonic *Globigerina angiporoides*. Also shown are calcareous nannoplankton zones designated as NP and NN Zones^{22,25} and planktonic foraminiferal zones^{25,35}. Eocene/Oligocene boundary is placed at the last appearance of *Globigerinatheka index*.

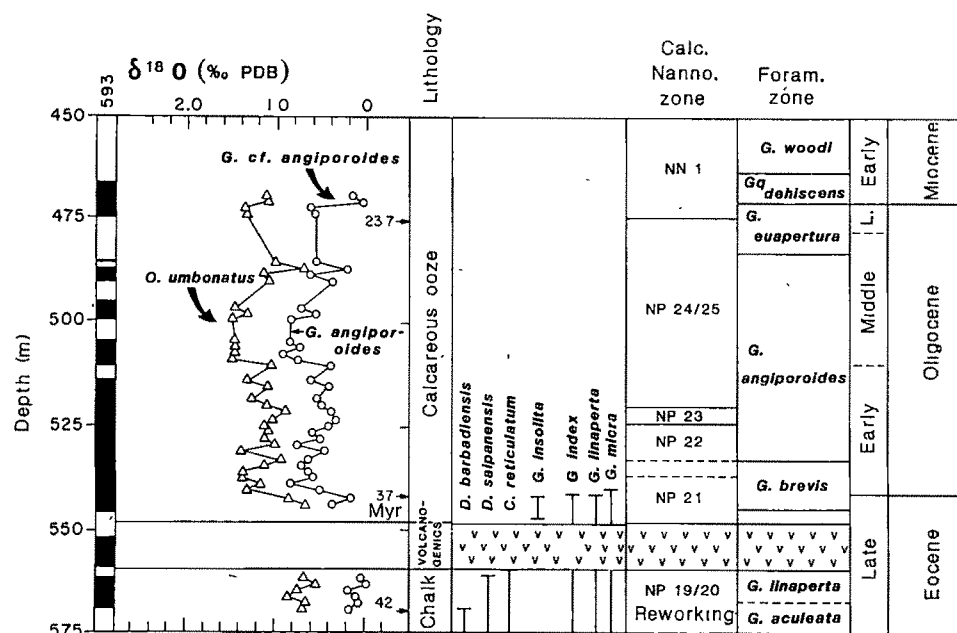
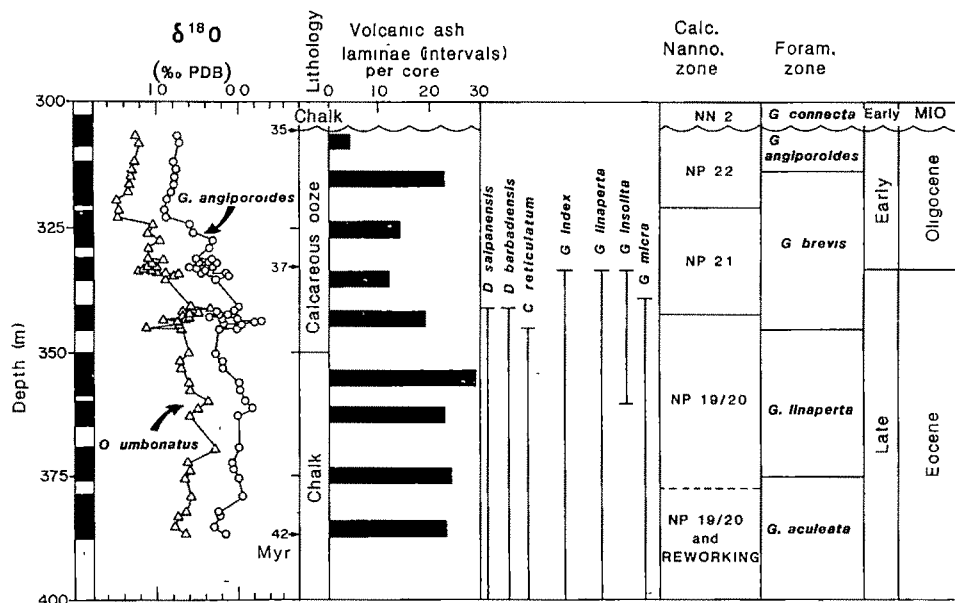


Fig. 3 Late Eocene through early Miocene biostratigraphy and oxygen isotopic stratigraphy of DSDP Site 593 and stratigraphical position of 15-m volcanoclastic unit of latest Eocene age. Oxygen isotopic curves are for the benthic foraminifer *Oridorsalis umbonatus* and the planktonic foraminifera *Globigerina angiporoides* and *G. cf. antiporoides*. Also shown are calcareous nannoplankton zones designated as NP and NN Zones^{22,25} and planktonic foraminiferal zones^{25,35}. Eocene/Oligocene boundary is placed at the last appearance of *Globigerinatheka index*.

material (Fig. 3). A radiometric determination of the volcanic material was not possible because of extreme submarine alteration (I. McDougall, personal communication); however, a latest Eocene age for the volcanics is consistent with the reversed geomagnetic polarity of the volcanoclastic sequences that suggests deposition during Magnetic Subchron C13R at ~37 Myr (refs 20, 21).

The $\delta^{18}\text{O}$ record (Fig. 3) exhibits a clear shift of 1.0‰ beginning in the late Eocene, just below the volcanoclastic unit, and reaching a maximum in early Oligocene Zones NP21/22. A gradual 0.5‰ decrease in early Oligocene $\delta^{18}\text{O}$ values was followed by a stepped increase of 0.5‰ in the late/early Oligocene; the $\delta^{18}\text{O}$ values gradually decrease 0.6‰ during the middle Oligocene²⁴. The volcanogenic unit was therefore emplaced during the well-known oxygen isotopic shift that marks the terminal Eocene.

Increased New Zealand volcanism

The pale green volcanic laminae, so abundant in the late Eocene/early Oligocene in Site 592 and in the late Eocene of Site 593, represent deposits resulting from highly active explosive volcanicity in the south-east Tasman Sea region. The volcanoclastic unit of latest Eocene age in Site 593 was derived from basaltic eruptions on the sea floor, with most of the massive

material mass-emplaced by pyroclastic or debris flows. The well-bedded and normally graded units were probably redeposited and sorted downslope by turbidity currents. Following deposition, the predominantly glassy sediments were extensively metasomatized and hydrated by the action of low-temperature seawater or by weathering phenomena, as shown by the abundance of smectite and phillipsite^{26,27}.

The Lalitha Pinnacle, the probable source of the latest Eocene volcanoclastic deposits, is one of several small pinnacles observed on seismic profiles on the Challenger Plateau²⁸ and on the southern Lord Howe Rise^{27,29}. These pinnacles were previously considered to have been formed between the middle Eocene and middle Miocene²⁹. Morphological similarity and the position of the other pinnacles in the seismic profiles suggests that the pinnacles are also of late Eocene age. The widespread nature of these volcanic centres and the abundance of altered volcanic ash layers in the late Eocene/early Oligocene sequences in comparison with the absence or rarity in the remainder of the Cenozoic clearly indicates intensified volcanism at this time in the New Zealand region. This conclusion is supported by the evidence for widespread volcanism of similar age in uplifted sections on New Zealand³⁰. These include the late Eocene basaltic Waiareka Volcanic Formation of north-east Otago³¹; the late Eocene/earliest Oligocene section of the Kekerione

Group of the Chatham Islands³²; and, in part, the Matakaoa Volcanics of East Cape³³. The basaltic Tangihua Volcanics of Northland are, in part, of late Eocene age, although their stratigraphical range is much older³⁰. The Tangihua and Matakaoa Volcanics have been interpreted as ancient seamounts³⁴. In north-east Otago, the Deborah Volcanic Formation (alkaline basalts, tuffs and agglomerates) was deposited within the *G. brevis* Zone and after the extinction of *G. index*^{35,36}, and thus is of earliest Oligocene age. North Canterbury and south Marlborough contain a fairly widespread submarine volcanic episode approximately coeval with the Deborah Volcanics, including the Cookson Volcanics consisting of widespread tuffs and less widespread basaltic pillow lavas^{30,37}. Much of this late Eocene/earliest Oligocene volcanism is localized and has been interpreted to be submarine in origin^{30,34}. In general, the Palaeogene of New Zealand, with the notable exception of the middle/late Eocene, represents a time of relative volcanic quiescence³⁰. The late Eocene also recorded the establishment of rapid subsidence and infilling of sedimentary basins in western South Island that resulted from locally increased tectonic activity^{38,39}.

Tectonic significance

The increase in late Eocene/early Oligocene volcanism and tectonism coincided with and was related to a change at 38 Myr in the pole of rotation that caused the development of an active tectonic zone through New Zealand^{38,40,41}. This active tectonic zone developed in association with the link-up of the South Pacific Rise with the south-east Indian spreading axis between Australia and Antarctica^{41,42}. A northward propagating zone of extension developed from the spreading ridges south of New Zealand along the western part of New Zealand as a linear zone of subsiding basins³⁹. Right-lateral movement may have developed along this zone as it linked up with basins to the north of New Zealand⁴¹, thus forming an extensional transform boundary across the New Zealand plateau⁴⁰. This effectively represented the development of an Indo-Australian/Pacific plate boundary in the latest Eocene^{38,43,44}. Increased explosive volcanism recorded in late Eocene sediments over wide areas of southern Australia has been linked to plate tectonic developments between Australia and Antarctica⁴⁵. Also an active phase of marginal basin development in the south-west Pacific marginal basins north of New Zealand⁴⁶ and eruption of basalts in New Caledonia during the late Eocene/early Oligocene⁴⁷ may have been associated with the development of this plate boundary. As yet, it is not clear as to the causal relations of the known New Zealand volcanism to the plate boundary, as this is located on either side of the main locus of deformation related to plate boundary development, rather than the eventual line of the boundary.

Circum-Pacific orogeny and volcanism

There is evidence that the pulse of volcanic activity during the late Eocene/earliest Oligocene in the south-west Pacific may have occurred throughout the circum-Pacific region. Pulses of explosive volcanicity have previously been recognized during the Neogene in the Circum-Pacific⁴⁸ and the Cenozoic in the North Atlantic⁴⁹. In Japan, there was a pulse of volcanicity during the late Eocene/earliest Oligocene (41–36 Myr)⁵⁰ related to termination of the Shimanto Orogeny^{51–53} and to initiation of the Mizuho tectonic phase in north-east Japan⁵⁴. In the Philippine Basin, there was a late Eocene pulse of island-arc related volcanism⁵³; and the Peruvian Andes show an important increase in volcanism during the late Eocene (~40 Myr) followed by a period of quiescence through most of the Oligocene⁵⁵.

Although we believe that the late Eocene volcanism in the New Zealand region was related to the development of a plate boundary through the area at that time, the Pacific-wide increase in volcanism was probably associated with a broader series of late Eocene plate-tectonic changes. The greatest late Phanerozoic change in Pacific Plate motion occurred during the late Eocene at ~42 Myr. This is most conspicuously reflected by the

bend in the Hawaiian–Emperor volcanic chain, located near the Yuryaku and Kanmu seamounts. Radiometric ages provide an age of these seamounts, and thus the bend, as between 41 and 43 Myr with the best weighted average of 42.3 ± 1.6 Myr (ref. 56). At this time, the volume of erupted volcanic material increased in the chain⁵⁷. The change in Pacific Plate motion is also recorded by a pronounced change in direction of the Mendocino Fracture Zone reflecting reorientation in spreading between the Pacific and Farallon Plates⁵⁸. SEASAT altimeter data exhibit a change in direction of the Eitanin and Udintsev Fracture Zones, reflecting plate motion change between 35 and 45 Myr, close to the age of the Hawaiian–Emperor bend⁵⁹.

Following this change in rotation of the Pacific Plate, there was an active phase in marginal basin development in the south-west Pacific, overthrusting of New Caledonia ultramafics and formation of new island-arc systems^{46,60}; increased late Eocene orogeny in Japan⁵⁴; and the initiation of the Philippine, Bonin, Mariana, Yap, Palau and Tonga trench-arc systems along former north-south trending fracture zones during the late Eocene⁶¹. In south-east Asia, there was a period of plate reorganization during the latest Eocene and early Oligocene⁶², and the Indian Ocean showed a coeval increase in spreading rate on the south-east Indian Ridge⁶³ as well as major change in spreading directions on both the south-west Indian Ocean and the Central Indian Ridges⁶⁴.

Turning points in the tectonic development of the Earth's major orogenies occur contemporaneously with first-order readjustments in plate tectonic motions because of the geotectonic-mechanical connections between the lithospheric plates^{65,66}. Each major change in the interlocking global stress system of plates may have global effects in mobile zones and may explain the near simultaneous increases in tectonism and volcanic activity⁶⁵. The northward motion of the Indian plate has slowed down from about 10 to 5 cm yr⁻¹ since the collision between India and Eurasia commenced at about 40 Myr (ref. 67). Also, the late Eocene (42–38 Myr) was a time of intense orogeny as represented by the Illyrian (Pyrenean) orogenic phase in the north Atlantic and Mediterranean regions^{65,66}. The Illyrian phase may have resulted from an abrupt change in the relative movement between the African and Eurasian plates. Such orogenic episodes have alternated with periods of relative tectonic quiescence^{65,69}.

Palaeoenvironmental significance

The late Eocene to earliest Oligocene was one of the most important intervals of global climatic cooling during the Cenozoic, especially at high latitudes^{3,4,13}. Increased biotic extinctions resulted from these environmental changes, although, unlike those associated with the terminal Mesozoic event, they were not of massive (catastrophic) proportions⁵ and the planktonic microfossil extinctions seem to have been concentrated in the extratropical areas where the cooling occurred. Several mechanisms, both terrestrial and extraterrestrial, have been proposed to explain the biotic and climatic changes that occurred during the terminal Eocene event^{3,70–75}. The problem of the causes of the terminal Eocene event is not without parallel with that of the terminal Mesozoic event, since catastrophic, extraterrestrial mechanisms have been invoked for each^{72,73,76}. Furthermore, the terminal Mesozoic event has recently been linked to a major increase in global volcanic activity⁷⁷. We believe that global climatic cooling during the terminal Eocene event, associated with increased Antarctic glacial development was created by fundamental changes in ocean circulation resulting from plate tectonics^{3,24}. Increased late Eocene explosive volcanism, related to the changes in plate tectonics, may also have contributed to this climatic cooling⁷⁸.

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A prolactin-inhibiting factor within the precursor for human gonadotropin-releasing hormone

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The cloned complementary DNA sequence encoding the human gonadotropin-releasing hormone (GnRH) precursor protein was used to construct an expression vector for the bacterial synthesis of the 56-amino acid GnRH-associated peptide (GAP). GAP was found to be a potent inhibitor of prolactin secretion and to stimulate the release of gonadotropins in rat pituitary cell cultures. Active immunization with peptides corresponding to GAP sequences led to greatly increased prolactin secretion in rabbits.

MAMMALIAN reproduction is centrally controlled through the synthesis and release of the decapeptide gonadotropin-releasing hormone (GnRH) by hypothalamic neurosecretory cells. The pulsatile secretion of this peptide into the vascular system connecting hypothalamus and pituitary triggers the episodic release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from gonadotrophic cells in the anterior pituitary^{1,2}. Additional, but less well understood, reproductive functions of GnRH are suggested by its synthesis in tissues such as gonads, placenta, mammary glands and extrahypothalamic regions of the central nervous system³⁻⁷.

That such synthesis in diverse tissues is the product of the same gene in human and rat has been established recently in our laboratory using recombinant DNA technology. Thus, complementary DNA clones containing coding sequences for a

GnRH precursor protein of relative molecular mass 10,000 (M_r) have been isolated from libraries derived from human placental⁸ and from human and rat hypothalamic messenger RNA⁹, and genomic analyses in both species revealed the presence of a single gene encoding this protein⁹.

The structure of the biosynthetic GnRH precursor as predicted from cloned nucleotide sequences⁸ comprises the decapeptide preceded by a signal sequence of 23 amino acids and followed by a Gly-Lys-Arg sequence necessary for enzymatic processing and carboxy-terminal amidation of GnRH. A sequence of 56 amino-acid residues occupies the carboxy-terminal region of the precursor and constitutes the GnRH associated peptide, GAP.

The central role of this precursor in reproduction and its resemblance to other peptide precursors from which multiple products having separate functions can be generated (see ref.

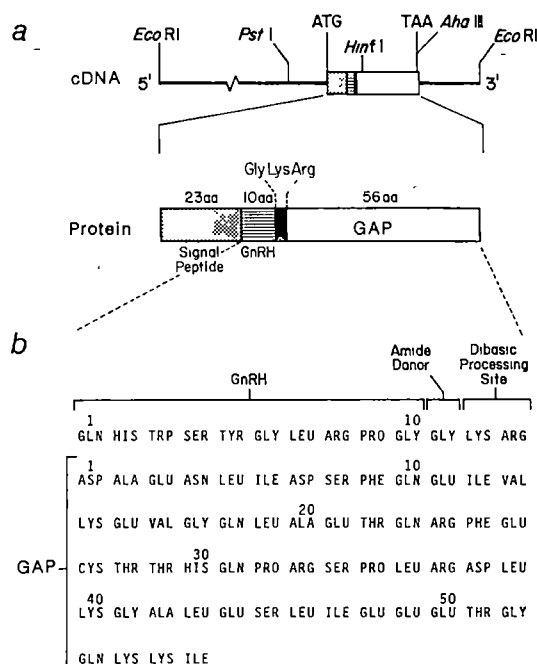


Fig. 1 Structure and encoded amino-acid sequence of human placental cDNA for prepro-GnRH⁸. **a**, Partial restriction endonuclease map. The coding region is located between the initiation codon for protein synthesis ATG and the termination codon TAA. Schematic representation of the encoded protein identifies the three domains of signal peptide, GnRH and GAP with the respective sizes in amino-acid (aa) residues. **b**, Amino-acid sequences of GnRH and GAP with an enzymatic processing site separating the two moieties. Numbers refer to the respective positions within GnRH(1-10) or GAP(1-56).

10 for review) had suggested the possibility that GAP is secreted together with GnRH to modulate pituitary function. Further evidence for this possibility was provided by our finding that antigenic determinants from GAP could be visualized in secretory vesicles in hypothalamic neurones, using immunocytochemistry¹¹.

As a step towards evaluating this possibility, we synthesized the 56-amino-acid peptide using recombinant DNA technology. A bacterial expression vector was constructed containing the coding sequences for GAP. This peptide was purified from bacterial extracts and tested for its activity on the secretion of anterior pituitary hormones using rat primary cell cultures. We report here that GAP inhibits prolactin release at subnanomolar concentrations and may represent the postulated peptide prolactin release-inhibiting factor (PIF)^{12,13}. GAP also displays gonadotropin-releasing activity with a concentration-response

curve similar to GnRH. Generation of gonadotropin-releasing and prolactin release-inhibiting activities from the same precursor protein may account for the known inverse relationship of LH/FSH and prolactin levels during certain physiological states of reproduction and lactation¹³⁻¹⁵.

Expression and purification of GAP

The structure of cloned human placental cDNA encoding the GnRH precursor is shown in Fig. 1, together with the deduced protein sequence. The coding sequences of this cDNA were used in the construction of a bacterial expression vector for the production of the 56-amino-acid GAP. As human GAP contains no methionine residues, we expressed this peptide as the carboxy-terminal part of a fusion with the *Escherichia coli* protein trpΔLE1413 (ref. 16) after introducing a methionine codon between the bacterial and human coding sequences. The presence of a methionine residue in this position allowed for the release of GAP by cyanogen bromide (CNBr) cleavage of the fusion protein produced by *E. coli*¹⁷.

As illustrated in Fig. 2, a DNA restriction fragment carrying the coding sequences for amino-acid residues 8-56 of GAP was isolated from plasmid DNA carrying the cloned cDNA and ligated to synthetic DNA encoding the first six amino acids of GAP preceded by a methionine codon and an *EcoRI* cohesive end. This ligation step also reconstituted GAP codon 7. An in-phase termination codon was created by cleaving the semi-synthetic DNA fragment with endonuclease *AhaIII* and inserting the resulting 177-base pair (bp) DNA into *EcoRI*/*HincII*-cleaved M13 mp18 replication form DNA¹⁸. The newly constructed GAP gene was isolated following primed DNA synthesis from recombinant M13 DNA as an *EcoRI*/*HindIII* DNA fragment and joined in-frame to the coding sequences of the *E. coli* trpΔLE1413 gene.

E. coli cells transformed with the resulting plasmid ptpLE-GAP were grown under conditions of tryptophan depletion and cell extracts were analysed for the presence of the fusion protein by SDS-polyacrylamide gel electrophoresis and immunoblots¹⁹ made using a GAP-specific antibody¹¹. The bacterial cells produced high levels of a GAP-immunoreactive protein of $M_r \sim 27,600$, a M_r expected from its genetically engineered size of 247 amino acids comprising 190 residues of the trpΔLE1423 protein plus the 57 amino acids of the Met-GAP peptide (Fig. 3B). *E. coli* cells containing the plasmid ptpLE-GAP were grown by high-density fermentation which yielded the fusion protein in the form of insoluble refractile bodies facilitating purification²⁰.

Protein purification was achieved by a combination of gel permeation and reverse-phase HPLC. The fusion protein was purified on a semi-preparative scale by reverse-phase HPLC with ~60% recovery from the refractile body preparation. CNBr treatment of this protein resulted in the release of GAP and additional fragments derived from the trpLE protein (Fig. 3A).

Table 1 Effect of GAP on the secretion of hormones from rat anterior pituitary cell cultures compared with basal levels and effects of known hypophysiotropic hormones

	LH	FSH	Prolactin	ACTH	GH
Basal	7.2 ± 0.59	2.56 ± 0.67	1,819 ± 193	66.2 ± 9.9	722 ± 112
GnRH	68.8 ± 6.52	11.2 ± 0.86	1,781 ± 351	74.6 ± 14.8	727 ± 67
TRH	ND	ND	3,032 ± 395	ND	ND
CRF	ND	ND	ND	287.1 ± 32.0	ND
GRF	ND	ND	ND	ND	3,080 ± 690
GAP	47.3 ± 5.16	7.3 ± 0.61	922 ± 106	73.2 ± 11.4	664 ± 117

Anterior pituitaries from female Sprague-Dawley rats (200-250 g) were dispersed by enzymatic treatment and cells kept in 24-well culture plates (5×10^5 cells per well) in medium 199 supplemented with 10% fetal bovine serum for 3-4 days when medium was changed as described previously^{31,32}. Incubations with the peptides GnRH, TRH, GRF (all from Peninsula Labs) and GAP were performed on the 4th or 5th day of culture in serum-free medium. 10^{-8} M concentrations of the peptides, except 10^{-7} M of TRH, were incubated with the cells for 4 h when media were removed and assayed for rat prolactin (PRL), LH, FSH and GH by double-antibody radioimmunoassay. Values are expressed in terms of recombinant PRL-RP-3, rLH-RP-2, rFSH-RP-2 and rGH-RP-2 reference proteins. ACTH was determined by radioimmunoassay as described previously³³ and is expressed in terms of pACTH. In a given cell culture, experimental points were determined from four parallel culture wells of which media were assayed by radioimmunoassay in duplicate. Values given for LH, FSH and prolactin are means ± s.d. of five independent cell cultures and those for ACTH and GH were from two cell cultures. All values are in ng per 5×10^5 cells $\times$ 1 ml medium $\times$ 4 h. ND, not done.

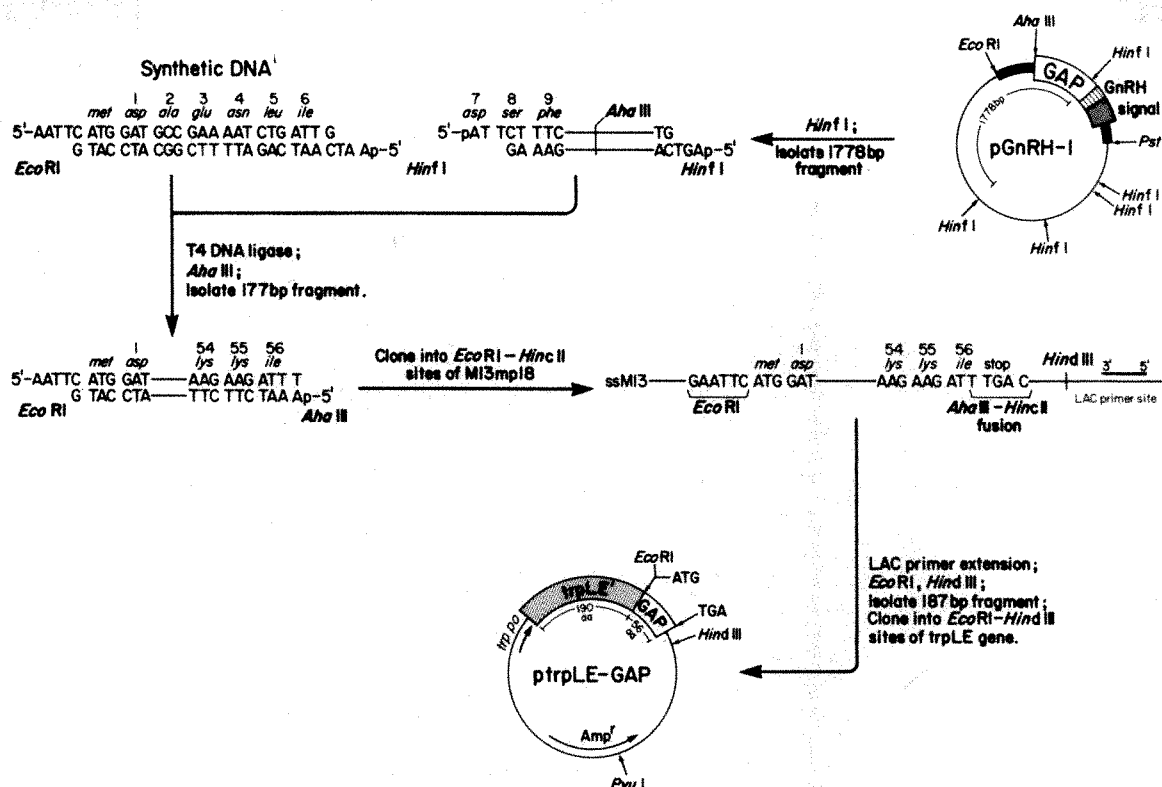


Fig. 2 Construction of plasmid *ptrpLE-GAP* DNA for the expression of GAP in *E. coli*. Amino-acid sequence numbers refer to positions in the GAP sequence.

Methods. The plasmid *pGnRH-1*, which contains the entire coding sequence for prepro-GnRH, was derived by subcloning a 568-bp *EcoRI*/*PstI* fragment from λ LHRH-1 (Fig. 1) into plasmid pUC11 (ref. 56). *pGnRH-1* DNA (15 μ g) was cleaved with *HinfI* and a 1,778-bp *HinfI* fragment was purified by polyacrylamide gel electrophoresis and subsequent electroelution. The two deoxyoligonucleotides, 5'-dAATTCATGGATGCCGAAATCTGATTG (fragment 1) and 5'-dAATCAATCAGATTTTCGGCATCCTAG (fragment 2), were chemically synthesized⁵⁷ and purified by gel electrophoresis. Fragment 2 (380 pmol) was phosphorylated at 37 °C for 30 min in a 20- μ l reaction containing 50 mM Tris-HCl (pH 8.0), 10 mM MgCl₂, 10 mM dithiothreitol (DTT), 1 mM ATP and 2 U T₄ polynucleotide kinase. The kinase was inactivated by heating the reaction to 100 °C for 3 min. Approximately 3 pmol (~3 μ g) of the 1,778-bp *HinfI* fragment was ligated to synthetic DNA in a 40- μ l reaction containing 50 mM Tris-HCl (pH 8.0), 10 mM MgCl₂, 10 mM DTT, 1 mM ATP, 150 pmol fragment 1, 150 pmol phosphorylated fragment 2 and 10 U T₄ DNA ligase. The ligation reaction was allowed to proceed for 2 h at ambient temperature before the ligase was heat-inactivated at 70 °C for 15 min. The DNA was then cleaved with *AhaIII* and electrophoresed on a 6% polyacrylamide gel. The correct 177-bp *EcoRI*/*AhaIII* DNA fragment was recovered by electroelution and ~7 ng was mixed with 10 ng *EcoRI*/*HincII*-cleaved M13 mp18 RF-DNA¹⁸ in a 20- μ l reaction containing 50 mM Tris-HCl pH 8.0, 10 mM MgCl₂, 0.1 mM EDTA, 1 mM ATP and 2 U T₄ DNA ligase. Transformation of JM101 cells using the ligated DNA produced many recombinant (white) plaques of which three were picked, grown and subjected to sequence analysis^{58,59} to confirm the correctness of the construction. Single-stranded mp18-GAP DNA was made double-stranded *in vitro* by elongating the 17-mer *lac* primer⁵⁹ in the presence of 1 mM deoxynucleotide triphosphates and *E. coli* DNA polymerase (Klenow). A 187-bp *EcoRI*/*HindIII* fragment was released from the resulting double-stranded DNA by *EcoRI*/*HindIII* cleavage and purified by polyacrylamide gel electrophoresis. The expression plasmid (*ptrpLE*, a derivative of pNCV-1 (ref. 20) containing a polylinker region and an ampicillin resistance gene) was cleaved with *EcoRI* and *HindIII* and ligated to the 187-bp *EcoRI*/*HindIII* fragment containing the GAP coding sequence. *E. coli* K-12 strain 294 cells were transformed with the ligation reaction and ampicillin resistant (*Amp*^R) colonies were screened by hybridization⁶⁰ for the presence of GAP sequences. Positively hybridizing clones were screened for their ability to synthesize a *trpLE-GAP* fusion protein (see Fig. 3). The sequence of one recombinant, *ptrpLE-GAP-26*, which produced the highest levels of fusion protein, was checked by supercoil sequencing⁶¹.

CNBr and formic acid were removed using reverse-phase HPLC and the cleavage products of the fusion protein were eluted by a step gradient. Given the significant size differences of these cleavage products, gel permeation chromatography was used to separate GAP from side products. The fraction containing the immunoreactive material was further purified by reverse-phase HPLC to homogeneity (Fig. 3C). Amino-terminal sequence analysis through 10 cycles confirmed the expected structure of the peptide (data not shown). Amino-acid analysis agreed with the theoretical composition of the molecule (see Fig. 3 legend). Polyacrylamide gel electrophoresis of purified GAP showed the presence of a single band immunoreactive after transfer to nitrocellulose (not shown).

Effect of GAP on hormone secretion

Primary cell cultures of the anterior pituitary have been extensively used to study the action of hypothalamic hypophysiotropic hormones. Recently, cultures of rat anterior pituitaries have aided the isolation and identification of corticotropin-releasing factor (CRF) and growth hormone-releasing factor

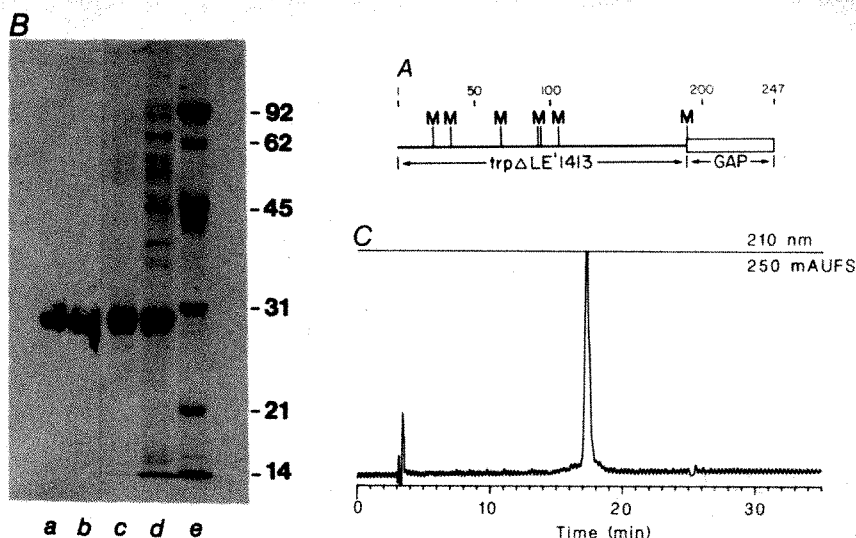
(GRF) of human and ovine origin²¹⁻²³. Using such cultures, we evaluated the effect of GAP on the secretion of LH, FSH, prolactin, corticotropin (ACTH) and growth hormone (GH). GAP was used at a concentration of 10⁻⁸ M, which for most hypophysiotropic hormones elicits maximal response from anterior pituitary cell or organ cultures. The effect of GnRH, thyrotropin-releasing hormone (TRH), CRF and GRF which stimulate gonadotropin, prolactin, ACTH and GH secretion, respectively, were also evaluated in parallel incubations. GAP did not affect the secretion of ACTH and GH but markedly influenced the secretion of LH, FSH and prolactin (Table 1). Although GAP stimulated the release of both gonadotropins, the peptide inhibited the basal secretion of prolactin by ~50%, comparable to inhibition levels reported for dopamine, a known prolactin-inhibiting factor^{24,25}.

Gonadotropin-releasing effect of GAP

Similar to GnRH¹, increasing concentrations of GAP caused an increase in both LH and FSH secretion from rat anterior pituitary cells, although maximal release was higher for GnRH

Fig. 3 Bacterial expression and purification of GAP. **A**, Schematic structure of the trpLE-GAP fusion protein indicating the location of methionines (M) and the GAP region. **B**, Analysis of total cellular proteins in *E. coli* K-12 strain 294 transformed with plasmid ptrpLE-GAP (**d**) and the purified trpLE-GAP fusion protein (**c**). Lanes **b** and **a** represent immunoblots¹⁹ of **d** and **c**, respectively. Relative molecular mass markers electrophoresed in parallel are shown in lane **e**. **C**, Analytical reverse-phase HPLC of purified GAP showing 210-nm absorbance profile. The peptide was chromatographed in conditions described below.

Methods. *E. coli* K-12 strain 294 cells containing the plasmid ptrpLE-GAP were grown in M9 medium containing 50 $\mu\text{g ml}^{-1}$ ampicillin. An aliquot of the cells was lysed in 2% SDS, 1% β -mercaptoethanol and precipitated with 10 vol of cold acetone. Samples were electrophoresed on 12.5% discontinuous SDS gels²² and proteins were either stained with Coomassie brilliant blue or transferred to nitrocellulose¹⁹. The latter was incubated with a GAP-specific antibody¹¹ and the cross-reacting protein visualized using horseradish peroxidase coupled to a second antibody followed by reaction with 4-chloro-1-naphthol¹⁹. For a 10-l high density fermentation culture, the plasmid ptrpLE-GAP was transferred to the *E. coli* K-12 strain W3110. Refractile bodies prepared as described previously¹⁷ were solubilized in 6 M guanidine hydrochloride containing 1% β -mercaptoethanol by vigorous stirring for 24 h at room temperature. The extract was then dialysed into 4 M urea and applied onto a Vydac C₄ 300 Å, 15–20 μm , 5 $\times$ 35 cm column and chromatographed using a gradient of 25–60% acetonitrile per 0.1% trifluoroacetic acid (TFA). Fractions containing GAP-27-40-immunoreactive material as tested by dot-blots⁶³ were pooled and lyophilized to yield 3.4 g fusion protein. 1.6 g of this material was subjected to CNBr cleavage and the reaction mixture, following dilution with 0.1% TFA, was chromatographed on a Vydac C₁₈, 300 Å, 15–20 μm , 2.5 $\times$ 35-cm column using a gradient of 30–50% acetonitrile containing 0.1% TFA. The fractions containing 6.200-M_r immunoreactive material were pooled and lyophilized to yield 285 mg crude GAP. This material was dissolved in 2 M acetic acid and subjected to gel filtration on a Sephadex G-50, 2.5 $\times$ 85 cm column eluting with 2 M acetic acid. 10 mg of the immunoreactive fraction (102 mg) was further purified by reverse-phase HPLC on a Vydac C₄, 300 Å, 5 μm , 1 $\times$ 25 cm column using a gradient of 33–45% acetonitrile/0.1% TFA to yield 1.3 mg GAP. Approximated amino-acid analysis, with theoretical values in parentheses: Asp, 4.65 (4); Thr, 3.98 (4); Ser, 2.99 (3); Glu, 13.67 (14); Gly, 3.20 (3); Ala, 3.31 (3); Val, 2.28 (2); Ile, 3.70 (4); Leu, 6.58 (6); Phe, 2.00 (2); His, 1.28 (1); Lys, 3.72 (4); Arg, 3.30 (3). Analytical HPLC conditions of **C**: Sample, 20 μg GAP in 25 μl 0.1% acetic acid; column, Vydac C₄, 300 Å, 5 μm , 0.5 $\times$ 25 cm; mobile phase, acetonitrile per 0.1% TFA; gradient: 36% to 44% in 30 min; detection: 210 nm at 0.25 absorbance units full scale (AUFS).



than GAP. Half-maximal stimulation of LH release by GAP occurred at higher concentrations than by GnRH (2×10^{-9} M for GAP compared with 8×10^{-10} M for GnRH). In contrast, GAP stimulated FSH secretion at approximately the same concentrations as GnRH with half-maximal effective dose (ED_{50}) values of the order of 5×10^{-10} M (Fig. 4b). There was no significant additive effect when cells were incubated with both GnRH and GAP.

Our finding that an additional peptide derived from the GnRH prohormone stimulates gonadotropin secretion has new implications for the potential selective regulation of LH and FSH release. Indeed, GAP alone shows a definite preference for stimulating FSH secretion as reflected in the ED_{50} values of LH (2×10^{-9} M) and FSH (5×10^{-10} M) release by the peptide (Fig. 4). The cause and physiological significance of this differential stimulation are unknown. As the simultaneous presence of GnRH and GAP in equimolar amounts failed to show significant differences in LH and FSH secretion, it is tempting to speculate that a differential release of the two peptides from hypothalamic neurones may change the ratio of circulating gonadotropins observed in certain physiological states².

Prolactin-inhibiting effect of GAP

The inhibitory action of GAP on the secretion of prolactin from pituitary lactotrophs *in vitro* was further investigated in terms of time- and concentration-dependence (Fig. 5). Highly effective concentrations of the peptide (10^{-10} – 10^{-8} M) caused maximal levels of inhibition to 40–45% of the basal secretion after 3–4 h of incubation (Fig. 5a). The gradual decrease in inhibition at longer incubation times could result from degradation of the peptide or may reflect desensitization. Based on these results, we evaluated the prolactin-inhibitory effect as a function of GAP concentration using 4-h incubation periods. The peptide was found to be active at very low concentrations with a half-maximal inhibitory dose value of 2.5×10^{-11} M (Fig. 5b). The maximal degree of inhibition by GAP was comparable with that

caused by 10^{-7} M dopamine. When tested simultaneously at different molar ratios, GAP and dopamine did not act synergistically (data not shown). When applied together with 10^{-7} M TRH, which considerably stimulated prolactin secretion (Table 1), GAP at 10^{-9} M exerted the same magnitude of inhibition as in the absence of TRH (data not shown).

Note that the effective concentration range for the prolactin release-inhibiting effect of GAP is an order of magnitude lower than the corresponding gonadotropin-releasing effect, suggesting that receptors on lactotrophs are more abundant or have a higher affinity for the peptide than receptors on gonadotrophs. As lactotrophs are more abundant in the anterior pituitary than gonadotrophs²⁶, it is likely that most peptide molecules interact with lactotrophs. As prolactin inhibition seems to be the primary action of GAP, the peptide is here classified as PIF.

To assess the physiological importance of GAP in controlling prolactin secretion, serum levels of this pituitary hormone were measured in six rabbits during 13 weeks of immunization with two synthetic peptide antigens corresponding to the amino-terminal and middle regions of GAP. Two animals immunized with unrelated peptide antigens served as controls. GAP-related immunization resulted in increased prolactin levels in all six animals, with most showing dramatic changes, whereas no significant change was seen in the controls (Table 2). GH levels were unaffected in all rabbits. For each animal the increase in prolactin levels could be correlated with the ability of the corresponding antibodies to recognize GAP itself as determined by immunocytochemistry and radioimmunoassay (K.N. *et al.*, in preparation). These data demonstrate that GAP has a dominant role in regulating pituitary prolactin secretion.

Discussion

It has long been recognized that prolactin secretion from the anterior pituitary is predominantly under inhibitory control¹². Although dopamine is known to exert such an inhibitory effect^{24,25,27–30}, there is evidence that dopamine cannot be solely

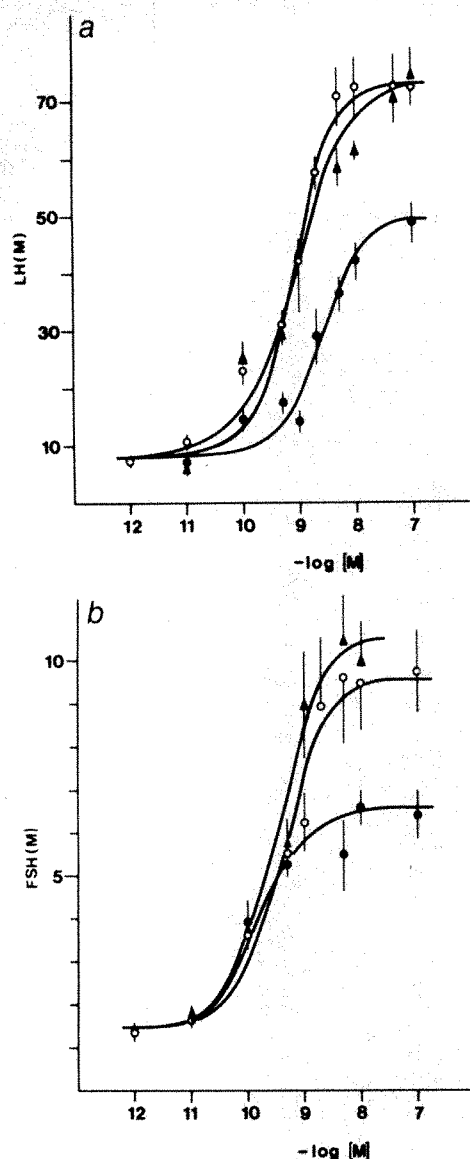


Fig. 4 Gonadotropin-releasing activity of GAP. LH (a) and FSH (b) secretion from rat anterior pituitary cell culture in the presence of increasing concentrations of GAP (●), GnRH (○) and an equimolar ratio of the two peptides (▲).

Methods. Anterior pituitary cell cultures were prepared as described in Table 1. The indicated concentrations of GAP, GnRH and their mixture were incubated with 5×10^5 cells per ml culture medium in 24-well culture plates for 4 h. Media were removed and assayed by radioimmunoassay. Values are expressed in terms of rLH-RP-2 (a) or rFSH-RP-2 (b) in ng per 5×10^5 cells. Each point represents the mean value of three independent cell cultures in which experimental points were obtained from four parallel culture wells of which duplicate samples were assayed by radioimmunoassay. Vertical bars, s.d. ED_{50} value of LH release induced by GAP was calculated to be 2.05×10^{-9} M with 95% confidence limits: lower, 1.02×10^{-9} M; upper, 7.05×10^{-9} M. ED_{50} value of FSH release induced by GAP was 5.02×10^{-10} M; lower, 9.95×10^{-11} M; upper, 1.18×10^{-9} M.

responsible for overall prolactin inhibition^{31,32}. The existence of a prolactin-inhibitory factor of peptidic nature has been invoked, but such a factor has not yet been characterized from hypothalamic extracts. Attempts to isolate hypothalamic PIF have shown that the areas containing dopamine-free PIF activity were located in the mediobasal hypothalamus and the organum vasculosum lamina terminalis³², regions that have many GnRH-producing and -secreting neurones³. Earlier purification studies from porcine and ovine hypothalamic tissue allowed for size estimates for this activity of $M_r < 8,000$ (refs 32–34).

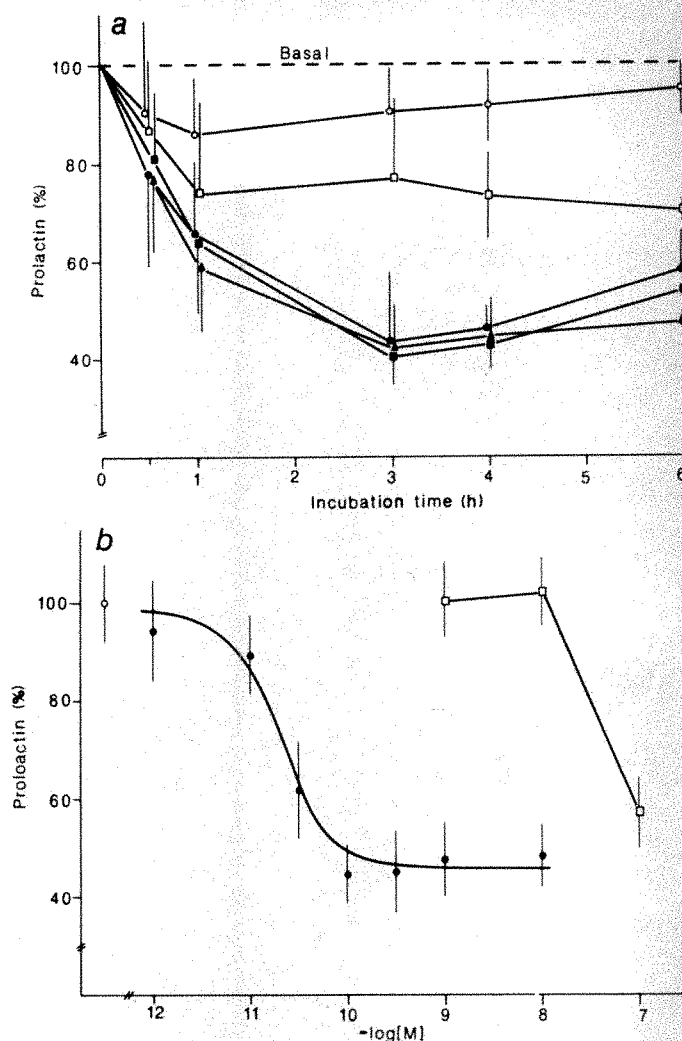


Fig. 5 Prolactin release-inhibiting effect of GAP. a, Prolactin secretion in the presence of 10^{-11} M (○), 3×10^{-11} M (□), 10^{-10} M (●), 10^{-9} M (■) and 10^{-8} M (▲) concentrations of GAP as a function of incubation time. Secretion is expressed as % of uninhibited basal levels. b, Prolactin secretion from rat anterior pituitary cell cultures in the presence of increasing concentrations of GAP (●) and dopamine (□). Control value without additions is shown by (○).

Methods. Anterior pituitary cell culture from female Sprague-Dawley rats were prepared as described in Table 1 legend. a, Different concentrations of GAP were incubated with 5×10^5 cells per ml medium for 6 h. Samples (20 μ l) of the media were removed at the indicated time points and assayed for prolactin. Values are expressed in terms of rPLR-RP-2 reference protein (National Pituitary and Hormone Program). Points are means of four parallel culture wells from which media aliquots were measured by radioimmunoassay in duplicate. Vertical bars, s.d. b, Indicated concentrations of GAP and dopamine were incubated with 5×10^5 cells per ml medium in 24-well culture plates for 4 h when media were removed and assayed by radioimmunoassay. Points are means $\pm$ s.d. of five independent cell cultures in which experimental points were obtained from four parallel culture wells of which duplicate samples were assayed by radioimmunoassay. Basal levels were determined from eight parallel wells in each culture. The curve shown for GAP was obtained by nonlinear fitting.

Using a different approach, we have here presented a peptidic PIF whose activity on lactotrophs is inhibitory for prolactin secretion at physiological concentrations and whose size and site of synthesis are as expected for the hitherto elusive hypothalamic PIF. This peptide of 56 amino acids was first described as the GAP located on a human placental GnRH precursor protein⁸. The amino-acid sequence for this precursor was deduced solely from the nucleotide sequence of a very rare cDNA species isolated twice from a cDNA library of $\sim 1.5 \times 10^6$ independent clones derived from human placental mRNA. The

Table 2 Effect of immunization with pro-GnRH(14-24) in rabbits 24A, B, C; pro-GnRH(33-56) in rabbits 56A, B, C and human insulin receptor (1-12) in rabbits 95, 96 (ref. 54) on serum prolactin levels

Weeks	Rabbits							
	24A	24B	24C	56A	56B	56C	95	96
0	440 ± 48	337 ± 6	141 ± 32	207 ± 33	434 ± 9	138 ± 10	277 ± 66	379 ± 39
6	2,417 ± 556	585 ± 117	870 ± 78	816 ± 33	586 ± 95	317 ± 33	339 ± 15	364 ± 13
7	7,843 ± 1,061	713 ± 31	1,379 ± 272	1,449 ± 42	ND	408 ± 60	278 ± 49	376 ± 10
9	6,126 ± 1,466	1,303 ± 442	4,920 ± 644	1,636 ± 52	444 ± 55	428 ± 68	533 ± 70	428 ± 7
10	5,190 ± 547	1,361 ± 187	4,851 ± 604	2,465 ± 353	662 ± 62	519 ± 69	501 ± 15	423 ± 21
12	4,515 ± 913	2,950 ± 116	5,084 ± 674	4,552 ± 684	756 ± 97	521 ± 83	497 ± 46	419 ± 23
13	4,826 ± 743	2,427 ± 491	7,939 ± 1,293	2,966 ± 616	1,309 ± 120	1,746 ± 192	531 ± 51	425 ± 16

Synthetic peptides were conjugated to soybean trypsin inhibitor by *N*-(maleimidobutyryloxy) + succinimide and injected intramuscularly into female rabbits at 0, 3, 5, 7, 10 and 13 weeks according to general procedures of immunization⁵⁵. Blood samples were taken at the indicated time points and assayed for prolactin and GH as described in Table 1. Values in ng ml⁻¹ are expressed in terms of rPRL-RP-3 reference protein and were obtained from duplicate determinations. ND, not done.

prediction of the amino-acid sequence of this peptide, and hence the elucidation of its pituitary function, was possible starting from a few grams of tissue, reflecting the use of recombinant DNA techniques in neuroendocrinology.

In this study we have used the complete 56-amino-acid carboxy-terminal peptide from pro-GnRH. Note, however, that the actual secreted form of this peptide into the hypothalamo-hypophyseal portal circulation is yet to be identified. The carboxy-terminus of GAP is Lys-Lys-Ile (Fig. 1), which may represent an enzymatic processing site giving rise to a 53-amino-acid form, yet a similar sequence at the carboxy-terminus of β -endorphin seems to be unprocessed³⁵. Although there is no further pair of basic amino acids in the molecule, the possibility that shorter fragments arise by cleavage at single basic amino acids cannot be excluded. However, the high potency of GAP suggests that the complete 56-amino-acid peptide is the secreted form and is required for full activity, illustrated by the case of GRF^{22,23}. This concept is also supported by our finding of highly elevated prolactin serum levels in rabbits immunized with synthetic peptide antigens corresponding to both the amino-terminal and the middle regions of GAP covering ~40 of the 56 amino-acid residues. Both synthetic peptides had negligible prolactin release-inhibitory activity *in vitro* (K.N. *et al.*, in preparation).

The fact that a prolactin-inhibiting peptide shares a precursor with GnRH suggests a new model for the hypothalamic control of gonadotropin and prolactin secretion. According to this model, the secretion of these pituitary hormones is coupled through the influence of the two hypothalamic peptides, GnRH and PIF, produced from a single precursor molecule expressed from a unique gene in a population of hypothalamic neurosecretory cells. Because of the differential actions of these peptides, our model predicts that gonadotropin and prolactin levels are inversely related. Thus, when hypothalamic neurones are triggered to secrete GnRH and PIF in a regular episodic pattern, circulating gonadotropin levels will be elevated and prolactin levels repressed. Conversely, irregular firing of the neurosecretory cells and low output of GnRH and PIF result in low gonadotropin and high prolactin release from the anterior pituitary. Whereas the former situation is predicted to occur during reproductive competence, the latter should govern lactation after parturition.

It is well understood that the control of PRL secretion is more complex than is suggested by the action of GAP, due to the influence of additional hypothalamic and extrahypothalamic factors on lactotrophs (see ref. 64 for review). Thus, for example, the co-surge of prolactin and LH found in certain physiological states could result from the overriding effect of prolactin-releasing factor(s)⁶⁵ and/or the stimulation of lactotrophs by factor(s) from GnRH-primed gonadotrophs⁶⁶. However, a wealth of physiological and biochemical evidence supports our model of a central concerted and inverse regulation of LH/FSH and prolactin by the concerted action of the two peptides derived from the GnRH precursor. Indeed, the early recognition of

concomitant inverse changes in gonadotropin and prolactin levels led to the belief that stimulation of gonadotropin release and inhibition of prolactin secretion might be two activities of the same molecule^{12,33}. Thus, suckling stimulates prolactin release but inhibits gonadotropin secretion, both effects being mediated by the hypothalamus^{36,37}. Reproductive disorders such as hypogonadism and amenorrhoea are often associated with hyperprolactinaemia, when pulsatile GnRH secretion is disturbed and low while prolactin is under insufficient inhibition³⁸⁻⁴⁰. The feedback action of oestradiol on the hypothalamus acutely increases prolactin and decreases LH secretion^{41,42}. The ontogeny of the hypothalamo-pituitary-gonadal system in humans and sheep is critically timed and correlates with prolactin secretion⁴³.

The first step is the development of the hypothalamo-hypophyseal GnRH-LH axis followed by the development of the negative feedback circuit via the hypothalamus from the gonads. The first appearance of the negative feedback effect of oestradiol on LH and FSH secretion on the 105th day in the ovine fetus has been found to be concomitant with an increase in prolactin secretion⁴⁴, evidence which is in good agreement with our model.

Opiates inhibit the secretion of GnRH⁴⁵ and the opiate antagonist naloxone increases LH and FSH levels with a concomitant reduction of prolactin secretion^{46,47}. A transplantable prolactin-secreting tumour was found to develop and act through the hypothalamus by inhibiting GnRH release paralleled by high prolactin output⁴⁸. In this study, low levels of LH and FSH were associated with very high prolactin levels despite increased portal blood levels of dopamine. Furthermore, γ -aminobutyric acid (GABA) displays PIF activity and an antagonist of GABA was found to suppress LH and FSH secretion through blocking GnRH output from the hypothalamus⁴⁹. Such GABAergic neurones are oestrogen-receptive and reside in the medial pre-optic/anterior hypothalamic area also controlling LH and FSH secretion⁵⁰.

In conclusion, a tightly coupled regulation of pituitary gonadotropin and prolactin secretion occurs, manifested through the hypothalamic production of a prohormone generating the two hypophysiotropic hormones GnRH and PIF. The fact that the same prohormone is synthesized in extrahypothalamic brain areas and in gonadal tissue suggests that GAP has additional central and paracrine functions involved in the regulation of reproduction.

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The murine T-cell receptor uses a limited repertoire of expressed V_{β} gene segments

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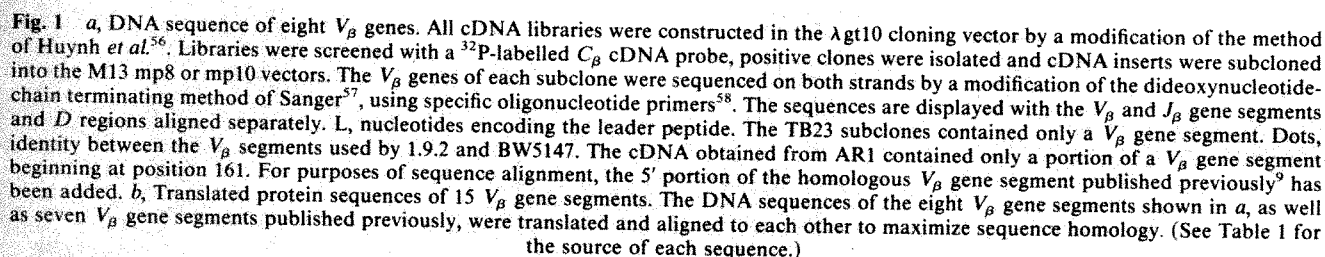
Only 10 different V_{β} gene segments were found when the sequences of 15 variable (V_{β}) genes of the mouse T-cell receptor were examined. From this analysis we calculate that the total number of expressed V_{β} gene segments may be 21 or fewer, which makes the expressed germline V_{β} repertoire much smaller than that of the immunoglobulin heavy-chain or light-chain genes. We suggest that β -chain somatic diversification is concentrated at the V_{β} - D_{β} - J_{β} junctions.

BOTH the T-cell receptor and the immunoglobulins are heterogeneous cell-surface glycoproteins that can recognize many antigens¹⁻³. It is clear from genomic analysis that they share similar strategies for diversification. T-cell receptor molecules are composed of α - and β -chains, each of which, like the immunoglobulin chains, is divided into variable (V) and constant (C) regions¹⁻³. The V regions together form the antigen-binding domain. The β -chain genes of the mouse are the most thoroughly studied T-cell receptor genes. Like the immunoglobulin genes, they are divided into separate V_{β} , diversity (D_{β}) and joining (J_{β}) gene segments that are assembled by recombination during T-cell development to form a V_{β} gene that is associated with either of two constant ($C_{\beta}1$ and $C_{\beta}2$) genes⁴⁻⁸. There are six functional J_{β} gene segments clustered just upstream of each C_{β} gene⁴⁻⁶ and two D_{β} gene segments, $D_{\beta}1.1$, upstream of the $J_{\beta}1$ cluster, and $D_{\beta}2.1$, upstream of the

$J_{\beta}2$ cluster^{7,8}. The total number of V_{β} gene segments is unknown. Like immunoglobulins, the T-cell receptor β -chain has at its disposal three sources of diversity: a multiplicity of germline gene segments⁴⁻⁹; combinatorial diversity through the assembly of different V, D and J segments^{4,9-11}; and somatic mutation^{9,11}. Immunoglobulin genes have three sources of somatic mutation: junctional flexibility at the sites of gene-segment joining¹²⁻¹⁴; the addition of random nucleotides to either side of the D-gene segment in the process of joining (N-region diversity)¹⁵; and somatic hypermutation^{16,17}. It is known that β -chain genes use the first two processes but may not use the third^{4,7-9,11}.

T-cell antigen recognition differs from that mediated by immunoglobulins in that T cells must recognize antigen in the context of a cell-surface molecule encoded by the major histocompatibility complex (MHC), a phenomenon termed MHC restriction^{18,19}. T-cytotoxic (T_C) cells, which are capable of killing virus-infected and tumour cells, are mainly restricted by class I gene products of the MHC²⁰. T-helper (T_H) cells, which are capable of enhancing B- or T-cell responses, are mainly

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We determined the nucleotide sequence of eight V_β genes obtained from cDNA libraries that were constructed from thymus cells, the T_H hybridoma 1.9.2 specific for the cytochrome *c* peptide AmDASp (ref. 22) and a functional T_C -cell line specific for MHC alloantigens. The sequence of these V_β genes and seven additional V_β genes taken from the literature were then compared (Fig. 1a, Table 1)^{4,9,11,23,24}. Of the 15 V_β genes analysed, 6 are from T_H cells, 2 from T_C cells, 6 from thymocytes and 1 from a T-cell tumour (Table 1). The antigen/MHC specificities of the functional cells are listed in Table 1. The specific functions and antigen/MHC specificities of the T-cell tumour and the cells that generated the thymocyte-derived V_β genes are unknown. As 99% of thymocytes do not migrate out

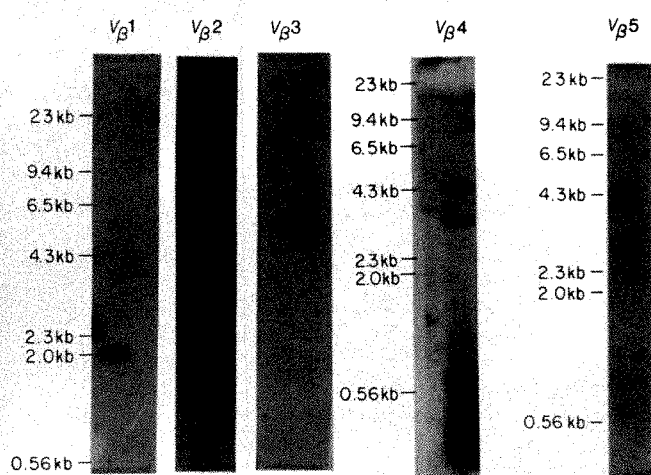


Fig. 2 Southern blot analysis of mouse germline DNA using probes from five different V_β gene segment subfamilies. BALB/c mouse liver DNA was digested with *Eco*RI ($V_\beta 1$, $V_\beta 4$, $V_\beta 5$ and $V_\beta 8$) or *Bam*HI ($V_\beta 2$). 10 μ g of each DNA digest was electrophoresed on a 0.8% agarose gel and transferred to nitrocellulose. Blots were hybridized with one of five ^{32} P-labelled V_β gene segment probes at 65°C in 1 M NaCl, 40 mM Tris, pH 7.5, 10% dextran sulphate, 1×Denhardt's solution, 0.1% SDS and 100 μ g ml $^{-1}$ denatured salmon-sperm DNA. The blots were then washed at 65°C in 0.15 M NaCl, 0.015 M sodium citrate, pH 7.5, and 0.1% SDS. The probes are derived from subclones of each V_β gene segment. The faint 5.7-kilobase (kb) band in the $V_\beta 2$ blot results from contamination of the probe with J_β region sequences.

of the thymus²⁵, some of the thymocyte-derived V_β genes may come from non-functional T cells. The 10 distinct protein sequences of the 15 V_β gene segments are shown in Fig. 1b: there are 10 distinct sequences. For reasons discussed below, the first seven sequences listed in Fig. 1b are designated $V_\beta 1$ –7 and the last three $V_\beta 8.1$, $V_\beta 8.2$ and $V_\beta 8.3$. One V_β gene segment appears three times ($V_\beta 1$), three appear twice ($V_\beta 2$, $V_\beta 3$, and $V_\beta 8.1$) and six appear once ($V_\beta 4$, $V_\beta 5.1$, $V_\beta 6$, $V_\beta 7$, $V_\beta 8.2$ and $V_\beta 8.3$). Moreover, when the sequences of seven additional V_β genes isolated from six T_H cells by our laboratory²⁶ and others are included (J. Gorman, unpublished data; M. Steinmetz, personal communication; A. Fotedar, P. Morinaga, B. Singh, T. Tamaoki and T. Wegmann, personal communication; S. Hedrick, personal communication), 11 different V_β gene segments are found in 22 rearranged genes. Six of the eleven different V_β gene segments appear more than once. Common V_β gene segments occur in functional T cells, thymocytes and

the T-cell tumour BW5147. The repeated use of V_β gene segments in a sample of this size suggests that the repertoire of expressed V_β gene segments is small. If we assume that the V_β gene segments are expressed randomly, the size of the V_β gene segment repertoire can be determined statistically.

If there are L species of V_β gene segments and they are expressed with equal probability and are randomly selected from the pool, then the probability (P) of obtaining an observed result is

$$P(m_1, m_2, \dots, m_N) = \frac{1}{L^N} \frac{L!}{(L-M)! M!} \times \frac{N!}{(1!)^{m_1} (2!)^{m_2} \dots (N!)^{m_N}} \times \frac{M!}{m_1! m_2! \dots m_N!}$$

where m_1 is the number of different species found once; m_2 , the number of different species found twice; m_N , the number of different species found N times; $N = \sum_{i=1}^N m_i$ = the total number of samples analysed; and $M = \sum_{i=1}^N m_i$ = the number of different species found in the sampling. Conversely, given the observed result, the relative likelihood that there are exactly L species in the pool is

$$P(L) = \frac{1}{\sum_{L'=M}^{\infty} \frac{L'!}{L'^N (L'-M)!}} \frac{L!}{L^N (L-M)!}$$

For the data discussed above, $N = 22$ and $M = 11$. By choosing a range of values for L and testing each for the probability of arriving at the value $M = 11$ for $N = 22$, the size of the expressed V_β gene segment family is 21 or less at the 95% confidence level. This estimate of the mouse V_β -gene segment repertoire is much smaller than the mouse immunoglobulin V_H (~100–300) or V_K (~100–300) gene-segment families^{27,28}, but larger than the mouse V_λ (2) repertoire²⁹. However, mouse λ -chains are expressed in only a few per cent of mature B cells, whereas β -chains are expressed in all T_H and T_C cells that have been analysed^{30,31}.

We do not know whether each V_β gene segment is expressed with equal probability in the T-cell population or if the sampling of V_β genes is random. In fact, the relative occurrence of V_β gene segments that we observe can also be explained by the frequent use of a small subset of V_β gene segments. Therefore, a much larger set of V_β gene segments could be expressed infrequently. However, the fact that we find identical V_β gene

Table 1 Characteristics and origins of sequenced V_β genes

V_β gene	Class	Strain	Antigen/MHC specificity	V_β	D_β	J_β	Ref.
2B4	T_H	B10.A	Cytochrome c/I-E ^{k} E ^{k}	3	2.1	2.5	4
1.9.2	T_H	B10.A[5R]	AmDASp/I-E ^{k} (see ref. 22)	1	1.1	1.1	*
3H.25	T_H	C57BL/6	Hen-egg lysozyme/I-A ^{b}	3	1.1	1.2	11
C5	T_H	C57BL/6	Dinitrophenol-ovalbumin/I-A ^{b}	8.1†	2.1	2.5	9
E1	T_H	BALB/c	Trinitrophenol/I-A ^{d}	2	1.1	2.2	9
LB2	T_H	C57BL/6	Chicken red blood cell/I-A ^{b}	6	2.1	2.3	9
HDS11	T_C	BALB.B	H-2 ^{d}	7	1.1	2.6	24
AR1	T_C	C57L	H-2 ^{d}	2	1.1	2.5	*
86T1	Thymocyte	BALB/c	—	1	1.1	1.3	23
TB2	Thymocyte	C57BL/Ka	—	8.2†	2.1	2.5	*
TB3	Thymocyte	C57BL/Ka	—	4	2.1	2.5	*
TB12	Thymocyte	C57BL/Ka	—	8.1	1.1 or 2.1‡	2.4	*
TB21	Thymocyte	BALB/c	—	5.1	2.1	2.6	*
TB23	Thymocyte	BALB/c	—	8.3†	ND	ND	*
BW5147	Tumour	AKR	—	1	2.1	2.5	*

* This paper.

† The three members of the $V_\beta 8$ -subfamily are denoted $V_\beta 8.1$, $V_\beta 8.2$ and $V_\beta 8.3$.

‡ So little of the D_β gene segment remains in the rearranged V_β gene that it is impossible to know which D_β gene segment contributed the sequence. ND, not determined.

segments expressed in T cells that differ in their antigen recognition and MHC restriction as well as between functional T cells and unselected thymus cells (Table 1), indicates that the effective repertoire of expressed V_β gene segments is probably very small. Accordingly, a large multiplicity of germline V_β gene segments does not seem to be a major contributing factor in the generation of T-cell receptor diversity.

Single gene subfamilies

Another method of estimating the size of the V_β gene segment repertoire is to determine the number of V_β gene segments in the mouse genome that cross-hybridize with V_β probes. This technique will identify V_β gene segments that have extensive homology with the available V_β gene segment probes, and gives a minimum estimate that can be compared with the predicted number. When this type of analysis was carried out with mouse V_H and V_κ gene segment probes, all the V gene segments fell into one of several distinct multigenic subfamilies^{27,28,32}. In mice there seem to be at least 7 V_H subfamilies ranging in size from 2 to >40 members and at least 5 V_κ subfamilies ranging in size from 2 to >20 members^{27,28,32}. (V gene segments with $\geq 75\%$ similarity have been defined as belonging to the same subfamily³³.) From this analysis it was estimated that the total repertoire of V_H and V_κ gene segments is ~100-300 members for each family^{27,28}.

Table 2 shows the percentage similarity between the 10 different V_β gene segments at the protein and nucleotide level. The nucleotide similarity of the V_β gene segments used by the TB2, TB12 and TB23 V_β genes range from 85 to 92%, indicating they are members of the same V_β subfamily. These V_β gene segments have been designated $V_\beta 8.1$, $V_\beta 8.2$ and $V_\beta 8.3$, respectively (Fig. 1b). The similarities among the remaining V_β gene segments range from 34 to 63%. Therefore, these V_β gene segments belong to different V_β subfamilies, which we have denoted $V_\beta 1$ - $V_\beta 7$. As the expressed V_β gene segment repertoire appears limited, it is important to have a generally accepted nomenclature for these sequences, as used with V_L and V_H subgroups³⁴.

To determine the size of the different V_β subfamilies, Southern blot analysis was performed on mouse liver DNA using DNA probes for the $V_\beta 1$, $V_\beta 2$, $V_\beta 4$, $V_\beta 5$ and $V_\beta 8$ subfamilies (Fig. 2). Three of the V_β probes show a single band, indicating that each V_β gene segment represents a different single-gene segment subfamily ($V_\beta 1$, $V_\beta 2$ and $V_\beta 4$). The $V_\beta 5$ and $V_\beta 8$ subfamilies appear to have two and three members, respectively (Fig. 2). Because the V_β gene segment used by the TB21 V_β gene is the first isolated member of the $V_\beta 5$ subfamily, we have denoted it $V_\beta 5.1$ (Fig. 1b). It has been previously reported that the $V_\beta 1$, $V_\beta 2$, $V_\beta 3$, $V_\beta 6$ and $V_\beta 7$ subfamilies are single genes and that the $V_\beta 8$ subfamily has two members^{9,11,24}. Thus, six different V_β subfamilies with one member, one V_β gene segment subfamily with two members and one V_β subfamily with three members have been identified. Including the additional V_β gene segment characterized by Malissen *et al.*²⁶, there are at least 12 mouse V_β gene segments. This minimum estimate for the size

of the V_β gene segment family falls within the range indicated by the statistical analysis presented above and is consistent with the hypothesis that the V_β gene segment repertoire is small.

The V_β gene segment family with its six single-member subfamilies differs from those of the immunoglobulin V_H and V_κ gene families, each of which contains 5 or more subfamilies with 2-40 or more members^{27,28,32}. Southern blots of DNAs from several rodent species as well as rabbit and human analysed with various V_β probes indicate that the single-member V_β subfamily sequences are less conserved between species than those of the three-member family ($V_\beta 8$)⁹. It has been suggested that this difference reflects selective pressures on the single-copy sequences to diverge rapidly, presumably to accommodate recognition of antigen in a changing MHC context⁹. An *ad hoc* argument for specific, positive selection must then be made to explain the unique conservation of the $V_\beta 8$ subfamily. In apparent conflict with this view is the observation that very little restriction enzyme polymorphism of single copy V_β sequences is seen between mouse strains that have diverged significantly in their MHC genes (B.S.K. and R.K.B., unpublished observations; D. Loh, personal communication). In addition, the inbred SJL mouse has deleted two of the five V_β gene segment subfamilies examined (B.S.K. and R.K.B., unpublished observations; D. Loh, personal communication). This indicates that mice have distinct V_β haplotypes containing different combinations of V_β subfamilies. Hence, an alternative explanation for the lack of V_β -gene segment conservation between species is that the ancestor to mammals contained different V_β haplotypes and during speciation distinct V_β haplotypes were passed on to different evolutionary lines. This model does not require a high rate of V_β gene segment mutation and consistent with the lack of restriction enzyme V_β polymorphism in mice. Additional data on the evolutionary divergence of the V_β gene segments should clarify the explanations for these observations.

It is unclear why single-member subfamilies seem to have arisen exclusively in the V_β gene family. Note that no V_β pseudogenes have been found, whereas at least 30% of V_H gene segments are pseudogenes²⁷. These observations raise a question about the mechanisms that are responsible for retarding the duplicative processes seen in other V gene families.

Diversification mechanisms

Despite the fact that a limited number of V_β gene segments have been identified, all 15 of the V_β gene sequences examined are distinct from one another because of combinatorial and somatic mutational processes (Fig. 1a)^{4,7-11}. The germline, combinatorial and somatic mutation contributions to V_β gene assembly are summarized below.

Germline. The β -chain gene family differs from its V_H counterpart in the apparently limited number of germline V_β and D_β gene segments, the expressed V_β gene segment repertoire consisting of perhaps 21 or fewer members compared with 100-300 germline V_H gene segments. In addition, although we do not know the number of germline D_β gene segments, comparisons

Table 2 Homology matrix of the 10 V_β gene segments

Subfamily	$V_\beta 8.1$	$V_\beta 8.2$	$V_\beta 8.3$	$V_\beta 6$	$V_\beta 7$	$V_\beta 1$	$V_\beta 3$	$V_\beta 4$	$V_\beta 2$	$V_\beta 5$
$V_\beta 8.1$	—	90	77	47	52	29	26	28	27	29
$V_\beta 8.2$	92	—	81	45	52	29	24	28	26	26
$V_\beta 8.3$	85	88	—	41	48	28	23	28	29	24
$V_\beta 6$	55	54	54	—	43	27	27	29	25	22
$V_\beta 7$	61	63	60	53	—	28	23	27	23	29
$V_\beta 1$	45	43	44	45	49	—	33	53	20	37
$V_\beta 3$	46	47	47	46	48	50	—	30	18	37
$V_\beta 4$	46	46	46	47	47	62	50	—	20	33
$V_\beta 2$	42	44	42	39	39	34	38	38	—	18
$V_\beta 5$	45	46	43	41	48	53	54	47	38	—

Numbers above the diagonal designate the percentage similarity of sequences on the x and y axes when compared at the protein level; numbers below the diagonal show percentage similarity at the DNA level.

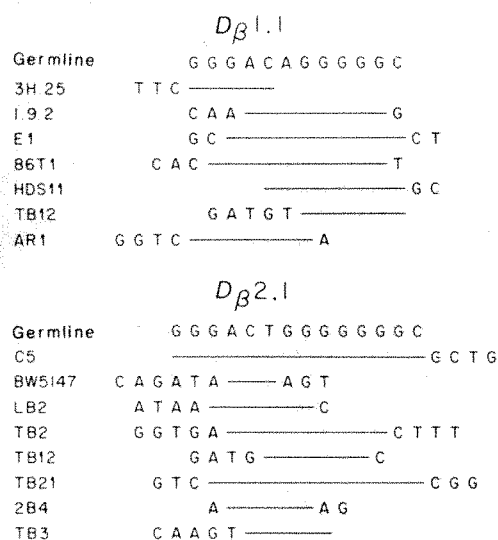


Fig. 3 The D regions from 15 V β genes may arise from just two D β gene segments. The D regions shown in Fig. 1a and those published previously^{4,9,11,23,24} were aligned to either D β 1.1 or D β 2.1. The regions homologous to either D β gene segment are represented by a straight line. The additional nucleotides flanking the germline D β sequences are presumed to be added by N-region diversity. The D region used by TB12 can be derived from either D β 1.1 or D β 2.1 and is so indicated.

of the D segments found in rearranged V β genes indicates that all could be derived from the two D β gene segments previously identified^{7,8} assuming extensive junctional flexibility and N-region diversification (Fig. 3). The heavy-chain locus, by contrast, has at least 10–20 D H segments³⁵. Finally, there are 12 apparently functional J β gene segments, 6 in each J β gene cluster^{4–6}, compared with 4 J H gene segments^{36–38}.

Combinatorial. Combinatorial joining permits either D β gene segment to be joined to any downstream J β gene segment (6 D β 1J β 1 + 6 D β 1J β 2 + 6 D β 2J β 2 = 18 D β -J β rearrangements). Individual V β gene segments appear to join any D β -J β rearrangements (21 $\times$ 18 = 378 V β genes). The D β 1 and D β 2 sequences are each used approximately half the time in the sample analysed and D β 1-J β 2 joinings occur as frequently as D β 1-J β 1 joinings (3 against 3) (Table 1). Eight different J β 1 and J β 2 gene segments are used, with J β 2 gene segments being used in 11 out of 14 examples. Thus, one would expect individual V β gene segments to join with the J β 1 gene cluster 25% of the time and the J β 2 gene cluster 75% of the time, which is what is found, supporting the contention that the joining of the D β 1.1 gene segment to either J β cluster occurs randomly. However, the J β 2.5 gene segment is used 6 of the 11 times that the J β 2 cluster is used. Whether this bias represents the selective effects of antigen or the mechanisms involved in DNA rearrangement is uncertain.

There may be two additional combinatorial mechanisms. Asymmetrical recognition sequences surrounding the D β gene segments potentially permit V β -J β and D β -D β joinings^{7,8}. Data consistent with the former possibility have been presented³⁹, although the interpretation of these data is difficult because of the possible loss of D gene sequences during V β gene segment rearrangement. As yet there is no evidence for either mechanism; hence, if these joinings do occur, they are infrequent.

Somatic mutation. Junctional flexibility in joining gene segments is illustrated in Figs 1a and 3. One to six extra nucleotides are found at either end of the D gene segments. Interestingly, there appears to be no G/C bias (50%) in this N-region diversification in contrast to that reported for immunoglobulin N-region diversity¹⁵.

The D β gene segments may join to the V β gene segments with equal probability in all three translational reading frames, with

the only requirement being that the D β -J β and V β -D β joinings leave the J β sequence in the proper translational frame (data not shown)⁴¹. In contrast, examination of >75 productively rearranged V H genes indicate that D H -gene segment families show a strong preference for joining in one translational reading frame (85%) (M. Kaartinen and O. Mäkelä, personal communication; T.H., unpublished observations). Presumably this difference arises as a consequence of more rigid structural or selective constraints on the D H as opposed to D β segments.

The somatic hypermutation of immunoglobulin genes occurs late in B-cell development, perhaps on exposure to antigen^{40,41}. By contrast, two V β gene segments expressed in functional T H cells specific for different antigens are identical to the germline sequence^{4,11}. On the other hand somatic variants can arise in alloreactive T cells *in vitro*⁴², although the physiological relevance of this observation is uncertain. We see two nucleotide substitutions and one replacement among the three V β 1, and two nucleotide substitutions and one replacement between the two V β 2 gene segments that have been sequenced (Fig. 1a, b)^{9,23}. As all these V β sequences are derived from different strains of mice (Table 1), these differences may result from polymorphism. No other differences were found and we tentatively conclude, in agreement with earlier workers^{4,11}, that somatic hypermutation, if it exists at all, is much less extensive in V β than in immunoglobulin V genes.

V-region similarities

It has been suggested that both the total diversity and the distribution of variability of V β segments differ from those of immunoglobulin V segments and that this may reflect the additional requirements for MHC recognition⁹. We have analysed the pattern of sequence diversity of the available V β sequences with this in mind.

The percentage similarities at the protein level (Table 2) between the different V β subfamilies ranges from 18 to 53% and 77 to 90% between members of the V β 8 subfamily. It has been suggested that V β segments are substantially more divergent than V H sequences⁹. Although their range extends to a lower value (18%) than that observed so far for known mouse V H segments (34%), or human V H segments (24%) (data not shown), when sampling biases are considered (see below) the maximum variation between different V β segments (82%) does not seem significantly larger than the maximum variation found between immunoglobulin V subfamilies (76%).

In immunoglobulin V regions, most of the sequence variability is clustered within three specific 'hypervariable' regions that form the antigen-binding crevice of antibody molecules^{43–46}. Two of these hypervariable regions are encoded by the V gene segments and the third is found in the V H -D H -J H or V L -J L junction regions⁴⁷. We have compared the pattern of variability of the 10 translated mouse V β gene segments (Fig. 1b) with that of a set representing all the known human V H segments and a set of 18 human V H segments with blocked α -amino groups (Fig. 4). Human V H segments were chosen for comparison because they offer a more random representation of V H sequences than any set of mouse V segments that has been sequenced. This is because mouse V H sequences have been highly selected in comparison with V β sequences in two ways. First, mouse V H sequences are derived mainly from immunoglobulins that recognize a relatively limited number of antigens. Second, for technical reasons, mouse V H sequences are almost exclusively determined from heavy chains with unblocked α -amino groups despite the fact that 80% of mouse serum immunoglobulins have heavy chains with blocked α -amino groups⁴⁸. The blocked human V H sequences, on the other hand, were selected randomly from various tumours and patients with other pathological conditions⁴⁷. We have found that the variability distribution of the mouse V β segments (Fig. 4a) is very similar to the distribution found for the set of human α -amino blocked V H segments (Fig. 4b). The variability distribution of the total set of human V H segments (Fig. 4c) represents a less random sampling than the human α -amino-blocked V H segments for the same reasons

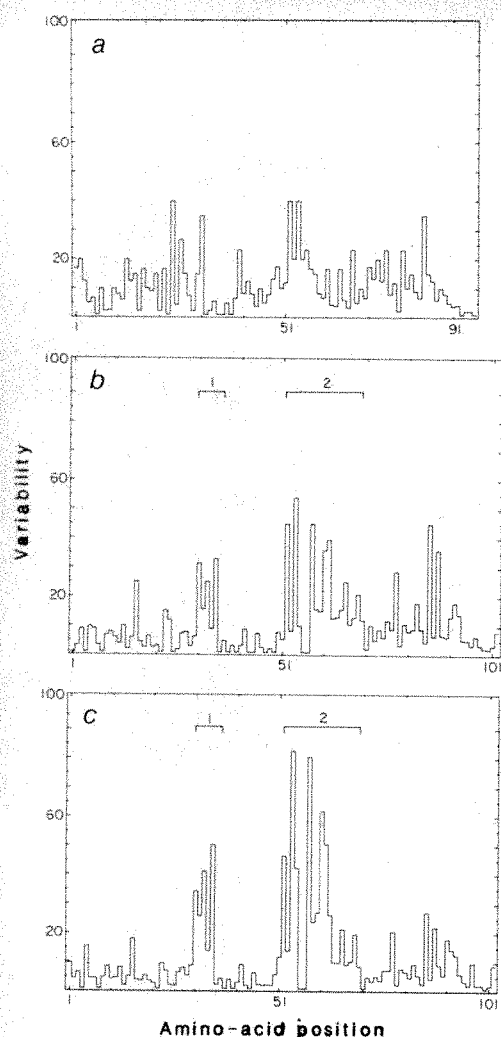


Fig. 4 Variability plot of V_β and V_H segments. Variability at each amino-acid position N is calculated⁴³ as: variability _{N} = no. of different amino acids that occur at N /frequency of most commonly occurring amino acid at N . Hypervariable regions can be defined empirically as a set of residue positions whose average variability is substantially greater than the mean variability of the entire sequence. The sequences used are all those available from the protein information resource of the National Biomedical Resource Foundation and GenBank. *a*, Translated sequence of the 10 distinct V_β gene segments shown in Fig. 1b; *b*, 18 α -amino-blocked human V_H sequences; *c*, 31 human V_H sequences representing sequences blocked and unblocked at the α -amino position.

given for the mouse sequences, and shows a more accentuated variability at the two classically defined hypervariable regions. The total set of human V_H sequences also exhibits a slightly lower background variability, partly resulting from the larger size of the sampling. Thus, it is critical to consider sample size and selection when this type of analysis is conducted. We conclude that the distributions of variability in the V_β and V_H segments are not significantly different from one another. Our results are not consistent with the suggestion⁹ that V_β regions have novel hypervariable regions relative to immunoglobulin V regions.

We have also compared V_β and immunoglobulin V segments by analysing them for two properties believed to reflect important structural features of these molecules, the distribution of β -pleated sheet-forming potential⁴⁹ and the predicted hydrophobicity profile⁵⁰. We find the results of these analyses to be almost identical for mouse V_β , V_H and V_κ segments (Fig. 5). Patten *et al.* have also reported that the β -pleated sheet patterns of several V_β segments conform to that of representative V_H and V_κ sequences⁹. V_β sequences also conserve essentially the

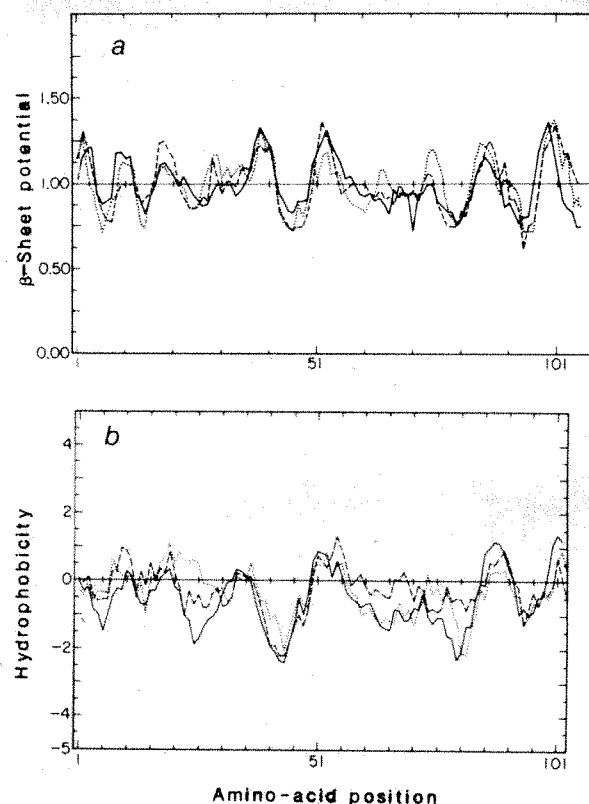


Fig. 5 Secondary structure analyses of V_β , V_H and V_κ segments. *a*, β -pleated sheet potential plots, using the method of Chou and Fassman⁴⁹; *b*, hydrophobicity plots using the scale of Kyte and Doolittle⁵⁰. Solid lines, β ; dotted lines, H ; dashed lines, κ . Analyses in *a* and *b* were based on the average value at each position for 55 V_H regions, 100 V_κ regions and 10 V_β regions.

same group of residues that in immunoglobulins are thought to be important in intra-chain structural interactions (data not shown)^{51,52}. These results strongly support the contention that the general biochemical characteristics and predicted secondary structures of the V_β , V_H and V_κ regions are very similar to one another. Accordingly, we predict that the T-cell receptor and immunoglobulin molecules fold into comparable tertiary structures. Therefore, from the analysis of the sequence variability and structural predictions, we find no evidence for the existence of any fundamental differences in how V_β gene segments can contribute to determinant recognition when compared with immunoglobulin V gene segments. This conclusion is supported by the observation that MHC-restricted antibodies have been raised to influenza antigens⁵³. This observation implies that there need be nothing structurally unique about the T-cell receptor structure and its ability to recognize antigen and MHC-restricting elements.

Antigen/MHC specificity

T cells can recognize a range of antigens similar to those recognized by B cells, but in conjunction with the entire range of polymorphic MHC molecules found in a species. Therefore, the T-cell receptor repertoire is expected to be at least equal to the immunoglobulin repertoire. Our observations suggest that the β -chain genes use fewer V gene segments than do immunoglobulins and that somatic hypermutation is infrequent or even non-existent. On the other hand, the V_β subfamilies exhibit the same overall range of sequence diversity as different V_H subfamilies. Furthermore, the contribution of J_β gene segments is three times that of J_H or J_κ gene segments, and because the D_β gene segments can be used readily in all three translational reading frames, the smaller family size than is seen in

immunoglobulins is at least partially compensated for. Finally, β -chains and immunoglobulins both use junctional flexibility and *N*-region diversification, which are major contributors to the generation of diversity within a specific region of the molecule involved in determinant recognition. Hence, we conclude that the limited number of expressed V_{β} gene segments and the infrequent use of somatic hypermutation does not necessarily reflect a restricted β -chain repertoire. Rather, these observations imply that β -chain somatic diversification is more focused to the 3' portion of the V_{β} gene.

There are three possible explanations for the apparent low frequency of somatic hypermutation of V_{β} genes. First, somatic hypermutation may act in B cells to increase the antigen binding affinity of antibodies rather than to generate a broader range of antigen response^{41,54}. T-cell receptors may not need somatic hypermutation because they may operate with a lower binding affinity due either to a lower affinity requirement for T-cell response or to the stabilizing effect of accessory molecules on the T-cell surface. Second, the current sampling may not include T cells equivalent to secondary-response B cells. Third, somatic hypermutation may have highly unfavourable consequences in T cells. In B cells somatic hypermutation occurs late in development, after antigen stimulation. It is possible that somatic hypermutation does not occur late in T-cell development, after immunocompetent cells have migrated from the thymus, in order to prevent the generation of autoreactive regulatory or cytotoxic T cells in the periphery. Thus, somatic diversification may be restricted to occur early in T-cell development, which allows the thymus to remove autoreactive cells arising from the somatic variation. B cells may not be so restricted because of the strong influence of regulatory T cells⁵⁵, and thus are free to undergo somatic hypermutation later in B-lymphocyte development in response to antigen stimulation.

As Table 1 demonstrates, there is no simple correlation between a particular V_{β} gene segment and distinct antigen specificities or MHC-restricting elements. For example, the $V_{\beta}2$ gene segment is used by a T_H -cell specific for trinitrophenol and the I-A^d MHC molecule (E1) and a T_C specific for the H-2D^d alloantigen (AR1). If the V_{β} and V_{α} regions fold in a manner similar to that of their immunoglobulin counterparts, as is suggested for V_{β} regions by our earlier analysis, then both chains will play a critical role in generating the binding site for antigen plus MHC. Accordingly, there is no reason to believe that either chain will have a particular role in recognizing either antigen or MHC individually.

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Note added in proof: Recently, the sequences of 15 additional V_{β} genes have been determined (D. Loh, personal communication). When these are added to the 22 existing V_{β} sequences, only 16 different V_{β} gene segments are defined from a total of 37 sequences. These additional data support our hypothesis of a limited V_{β} gene segment repertoire. Furthermore, when these data are included with our own data and analysed for the distribution of variability, hydrophobicity and β -pleated sheet-forming potential, the results are very similar to those presented here.

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Ultraviolet continuum variability of the quasar 3C273

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Several quasars show an excess of radiation in the ultraviolet over the power law defined by their infrared/optical continuum. Several authors¹⁻⁴ have fitted the infrared to ultraviolet spectrum of these quasars with a combination of a power law component, line emission, blends of Fe II lines, Balmer continuum and a black-body component which dominates the ultraviolet spectrum. We report here the first observations of variability in the ultraviolet spectrum of the quasar 3C273 (redshift, $z=0.158$) as observed by the International Ultraviolet Explorer (IUE)⁵ in the period 1978-84. The flux at $\lambda_{\text{observed}} = 1,675 \text{ \AA}$ increased by 1.25 between April and June 1982, then decreased by a factor 2 between June 1982 and April 1983. The amplitude of these variations and the constancy of the intensity of the Ly α emission line during the same period are indications that the ultraviolet variations are caused by variations of the black body and/or non-thermal components and not by variations of the continuum or blend of lines originating from the broad-line region. The flux variations are accompanied by variations of the spectral shape. We present an interpretation of the observed variability in terms of a discontinuous and variable distribution of the temperature on the photosphere emitting the ultraviolet continuum.

Archived IUE low-dispersion spectra of 3C273 taken between July 1978 and July 1984 have been analysed. These spectra were taken in various programs and were therefore not optimized for a study of the variability. We have retained only the images taken through the large aperture, and which are not overexposed, or only weakly overexposed, at the peak of the Ly α emission line (Table 1).

Standard IUE data reduction, IUESIPS, was performed on the raw data to obtain calibrated spectra from which daily averages (weighted by the exposure time) were made when several spectra on the same date were available. The flux f_ν was binned into 50- $\text{\AA}$ bins although only those bins were retained for which no reseau or emission line could modify the spectrum significantly. Ten bins were selected in the long wavelength (LW) range of IUE between $\lambda_{\text{obs}} = 2,310$ and $2,860 \text{ \AA}$. The FeII multiplets make a small contribution to the flux in all the bins. Wills *et al.*⁶ have studied the quasar-energy distribution in the

range 2,000-5,000 $\text{\AA}$, and find that the FeII spectrum contribution used in the fit of the spectrum of 3C273 does not exceed 0.15 of the total flux at any wavelength. In the short wavelength (SW) range, there are only two bins free of emission lines, centred at $\lambda_{\text{obs}} = 1,275$ and $1,675 \text{ \AA}$, or $\lambda_{\text{rest}} = 1,100 \text{ \AA}$ and $1,446 \text{ \AA}$, respectively.

The sensitivity of the IUE cameras is slowly decreasing with time in a wavelength dependent way^{8,9}. The sensitivity decline of the short wavelength prime (SWP) camera is $<1\% \text{ yr}^{-1}$ for $\lambda < 1,800 \text{ \AA}$ (the region of interest here). For the long wavelength redundant (LWR) camera, the decline has been by $\sim 2.5\% \text{ yr}^{-1}$ for $2,250 < \lambda < 2,550$ and $\sim 1.3\% \text{ yr}^{-1}$ for $\lambda > 2,550 \text{ \AA}$. We have made no correction for this and the error so induced is $<2\%$ for the SWP camera and $<5\%$ for the LWR camera for the period 1982-84. The r.m.s. error on individual flux measurements of optimally exposed spectra is 3.5% for all the three cameras. A further source of error of up to 3% is introduced by the different exposure times used at the different epochs. The r.m.s. of the error resulting from these different sources of error is thus $\sim 5\%$ for the SWP camera and 7% for the LWR camera in the period 1982-84. The LWR fluxes of 1978 and 1979 are overestimated by ~ 10 and $\sim 8\%$, respectively, with respect to the 1982 LWR fluxes, while the SWP fluxes of 1978 and 1979 are overestimated by 5 and 3%, respectively, with respect to the 1982 SWP fluxes.

Figure 1a shows the light curve of 3C273 at $\lambda_{\text{obs}} = 1,675 \text{ \AA}$. The flux is usually near $f_\nu \sim 13 \text{ mJy}$ except in the period April-June 1982 during which it increased from 18 to 23 mJy.

The ultraviolet energy distribution of 3C273 in the period April 1982 to July 1984, on which we concentrate our analysis,

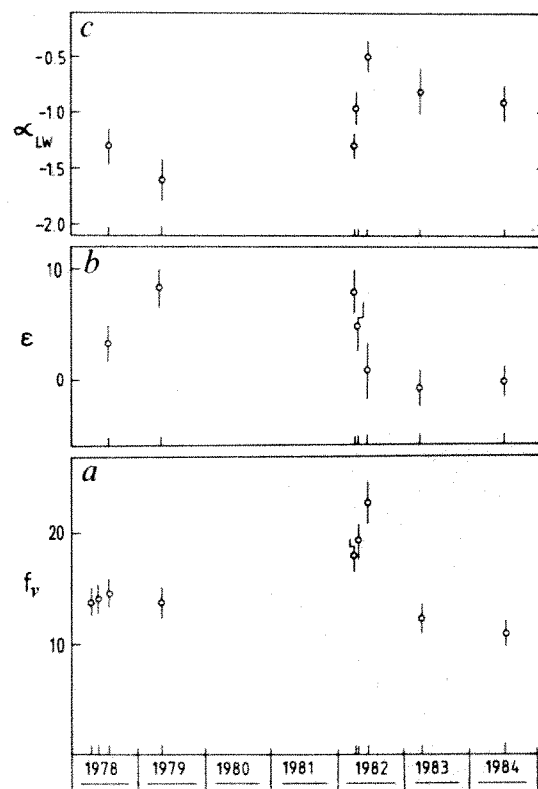


Fig. 1 a, The flux of 3C273 as a function of time averaged over a 50- $\text{\AA}$ bin centred at $\lambda_{\text{obs}} = 1,675 \text{ \AA}$. No reddening correction has been applied. Ordinates in mJy. b, The parameter ϵ represents at each epoch the difference between the SW flux near $1,460 \text{ \AA}$ and the value at $1,460 \text{ \AA}$ of the power law representing the LW spectrum. The values of ϵ have been calculated after a reddening correction, $E(B-V) = 0.05$, has been applied. Ordinates in mJy. c, α_{LW} is the spectral index of the power law representing the spectrum in the LW range after correcting the spectra for reddening $E(B-V) = 0.05$.

Table 1 Short and long wavelength spectra

Date	SW spectra	Exposure (min)	LW spectra	Exposure (min)
15 April 1978	SWP 1,366*	50		
26 May 1978	SWP 1,642*	90		
24 July 1978			LWR 1,886*	50
25 July 1978	SWP 2,099*	50	LWR 1,887*	40
4 May 1979	SWP 5,116*	25		
5 May 1979			LWR 4,454*	40
18 April 1982	SWP 16,790	3 × 40	LWR 13,042	3 × 40
20 April 1982	SWP 16,801	30	LWR 13,055	30
	16,802	30	13,056	30
	16,803	30	13,057	30
27 June 1982	SWP 17,300	15	LWR 13,562	20
	17,301	23	13,563	23
	17,302	27	13,564	22
	17,303	12		
15 April 1983	SWP 19,731	15	LWR 15,739	15
11 July 1984	SWP 23,444	39	LWR 3,757	30

* Reprocessed in 1984 at ESA Villafranca Satellite Tracking Station.

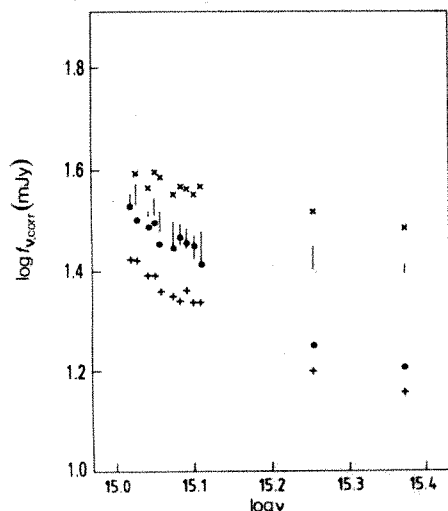


Fig. 2 The spectrum of 3C273 corrected for reddening, $E(B-V) = 0.05$, at five different dates between April 1982 and July 1984. $\times$, 27 June 1982; $|$, 18 and 20 April 1982; $\bullet$, 15 April 1983; $+$, 11 July 1984. The flux values have been averaged in 50-Å bins. In the short wavelength range of IUE, only the bins centred at 1,275 and 1,675 Å are free of emission lines. Blended Fe II multiplets contribute weakly and unevenly to the flux in the 10 long wavelengths bins selected between 2,310 and 2,860 Å. Ordinates in mJy. The two points which at each wavelength represent f_v for 18 and 20 April 1982 are joined by a vertical bar. The points of 18 April are always at the lower end of the bar except at $\lambda_{\text{obs}} = 1,275$ Å.

is shown in Fig. 2. A correction for reddening corresponding to $E(B-V) = 0.05$ has been applied. This value is the reddening in our Galaxy as deduced from observations of H I absorption in the direction of 3C273 (ref. 7). The dereddened spectral shapes discussed below depend on the choice of $E(B-V)$ but the differences between spectral shapes persist whatever the value of $E(B-V)$. The two points which at each wavelength represent f_v for 18 and 20 April 1982 have been joined by a vertical bar. The points of 18 April are always at the lower end of the bar except for the shortest wavelength bin centred at $\lambda_{\text{obs}} = 1,275$ Å. It is not clear whether this systematic difference is real and is part of the increase observed between April and June 1982, but it does represent an upper limit to the error on the reproducibility.

Figure 2 shows that the spectra vary in flux and in shape. Whereas the whole ultraviolet continuum can be reasonably well fitted by power laws of various slopes on most dates, the spectrum in April 1982 definitely cannot, the flux in the SW bins lying well above the extrapolation of the LW spectrum. We emphasize that the power laws are used here as simple, smoothly varying functions with no inflexion point, which, in some cases, provide an acceptable approximation of the data. Over the small-frequency range analysed here they can fit an optically thin synchrotron spectrum or the blue part of a black-body function or a combination of the two.

During the period 1982–84 the intensity of Ly α has not changed appreciably. This is illustrated in Fig. 3 which shows the spectra of 27 June 1982 (thick line) and 11 July 1984 (thin line) in the vicinity of Ly α . The spectra coincide after the 1984 spectrum has been shifted by $+18.0 \times 10^{-14} \text{ erg cm}^{-2} \text{ s}^{-1} \text{ Å}^{-1}$. Excellent theoretical calculations of line intensities in quasars and Seyfert galaxies have been published by several authors. The calculations by Kwan¹³ show that the intensity of Ly α is practically proportional to the value of the ionizing parameter. Moreover, the ratio of the intensity of the iron lines in the interval 2,240–2,650 Å over the intensity of the Ly α line does not increase by more than a factor ~ 2 for an increase by a factor 100 of the ionizing parameter. For this reason we take the large variations of f_v and the constancy of the Ly α intensity as an indication that the changes in 1982–1984 did not originate in the broad line region (line blends or Fe II continuum and free-free, free-bound H continuum) but were caused by variations

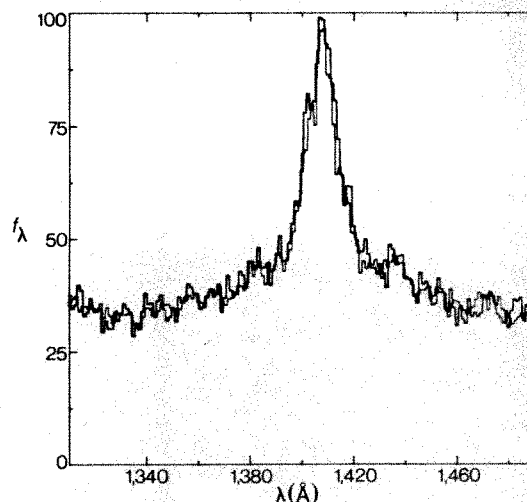


Fig. 3 The spectrum of 3C273 near the Ly α emission line. Thick line 27 June 1982 (average of SWP 17,301 and SWP 17,302). Thin line 11 July 1984 shifted by $+18 \times 10^{-14} \text{ erg cm}^{-2} \text{ s}^{-1} \text{ Å}^{-1}$. The continuum in June 1982 is 1.8 times stronger than in July 1984 while the Ly α line has not varied significantly. Ordinates in $10^{-14} \text{ erg cm}^{-2} \text{ s}^{-1} \text{ Å}^{-1}$. No reddening correction has been applied.

of the central black body and/or non-thermal component. The fact that Ly α has not been observed to vary is probably due to a time delay of months between the ionizing continuum source and the broad line gas.

A quantitative appreciation of the change in the spectral shape is obtained by introducing two parameters: (1) the slope, α_{LW} , of the power law fitting the spectrum in the LW range after the reddening correction has been applied. The values of α_{LW} are plotted in Fig. 1c. Although α_{LW} is smallest in June 1982 when the flux is highest the data available do not show a correlation between α_{LW} and flux unlike observations in some Seyfert galaxies, for example, NGC4151 (ref. 10). More numerous data covering a larger range of flux variations might, however, show such a trend. (2) To quantify the discontinuity in the slope of the ultraviolet spectrum occurring at some dates near 2,200 Å we use the parameter ϵ . Let us consider the point which, in the $\log f_v$ versus $\log v$ plot, is at mid-distance between the two points representing the flux at 1,275 and 1,675 Å. Its abscissa corresponds to 1,461 Å. The parameter ϵ is the difference between the ordinate of this point and the value at $\lambda = 1,461$ Å of the power law representing the LW spectrum (and having the slope α_{LW}). Figure 2b gives ϵ calculated from the dereddened spectra. A small value of ϵ means that the whole (dereddened) ultraviolet spectrum can be well represented by a power law. A large value of ϵ suggests the presence of two spectral components varying independently.

The variations of the ultraviolet continuum spectral shape observed in 1982 occurred at a time when the ultraviolet flux was approaching its observed maximum (Fig. 1a). The sparse time coverage of this event and the lack of correlated optical measurements make it difficult to calculate accurately the energy radiated in this 'flare'. The order of magnitude can be estimated assuming that the ultraviolet continuum flux stayed for ~ 2 months at its value of 27 June 1982 which is about twice its value in preceding and following years.

Taking the spectral decomposition of ref. 4, which is based on IUE data of 3C273 of 1978–79, gives an ultraviolet luminosity of $\sim 10^{47} \text{ erg s}^{-1}$. A flare lasting for about 2 months would, therefore, radiate $\sim 5 \times 10^{53} \text{ erg}$ ($H_0 = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$).

We note that the discontinuity in the ultraviolet spectrum at $\lambda \sim 2,200$ Å is not reproduced well by any published combination of various continua and smooth black-body spectra. This discontinuity, which is most apparent in April 1982, suggests that, in addition to the black-body component at 20,000–26,000 K recognized by several authors^{1–4}, there could be a hotter component with $T \sim 35,000$ –40,000 K covering a smaller area than the

20,000 K component. One can speculate that the spectral variations of April–June 1982 are due to an increase of the flux emitted by the hotter component (increase in area) probably accompanied by a smoothing of the temperature distribution. The hot component may result from the sudden heating of the inner part of an accretion disk. Another speculative possibility is the explosion of a supernova in a large cluster of stars coinciding with the central source. In the wind and shock model¹¹, this would cause a heating of one part of the ultraviolet photosphere.

The flare seen in the ultraviolet should manifest itself in other regions of the spectrum. Note that a flare in the millimetre-infrared was observed in 1983, although with a significantly lower energy, 10^{45} erg (ref. 12). It is uncertain whether the two flares are related. This points to the importance of continuous broad-band monitoring of this type of sources, as the observation of a time delay between the flaring at different wavelengths would give precise information on the relations between the different physical components.

The presence of flares in different parts of the spectrum makes the spectral decompositions attempted^{1–4} very dependent on the epochs of the measurements at different wavelengths. This effect has yet to be taken into account.

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Discovery of Jupiter's 'gossamer' ring

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Jupiter's ring system has previously been described as being composed of a 'bright' narrow ring and an interior, vertically-extended halo^{1,2}. The one image which reveals this morphology most clearly is Voyager 2's parting shot of the Jupiter system, a wide-angle (WA) view of the ring ansa in forward-scattered light (FDS 20693.02). The bright ring is plainly visible, and the halo appears after slight contrast enhancement. By further enhancement of this image we have discovered an additional ring, which is far fainter than either of the (already faint) components previously identified, extending to a radius of 210,000 km.

The ring is visible as the blue-green stripe extending outward from the bright ring in Fig. 1. Although this feature has a mean intensity of only one-half the overall image noise level, it covers such a large area ($\sim 3 \times 10^4$ pixels) that its integrated signal-to-noise ratio is ~ 110 . Previous WA frames were checked to verify that this is not a ghost image, such as are occasionally visible in Voyager data. The lack of systematic variations of this form in any other Voyager images, as well as the consistency of this feature's morphology with a flat circular ring in Jupiter's equator,

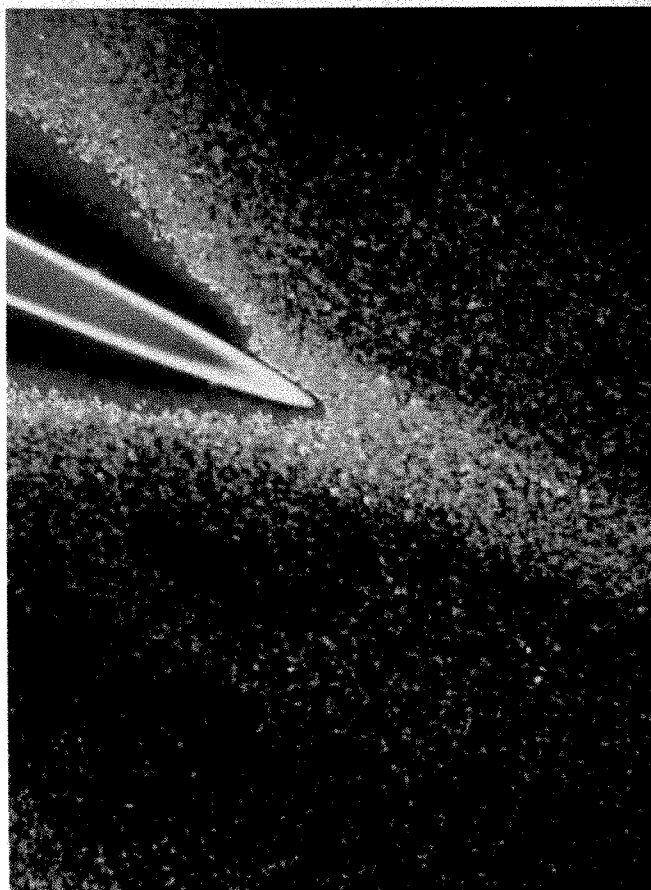


Fig. 1 An enhancement of Voyager image FDS 20693.02, shown black-and-white above and colour on the cover. Here discrete colours of the spectrum have replaced a range of the darkest shades of grey in the image. The newly-discovered 'gossamer' ring is visible as a blue-green stripe running outward from the bright ring (in white). The halo (outlined in red-yellow) rises at the bright ring's inner edge, and extends vertically above and below the ring.

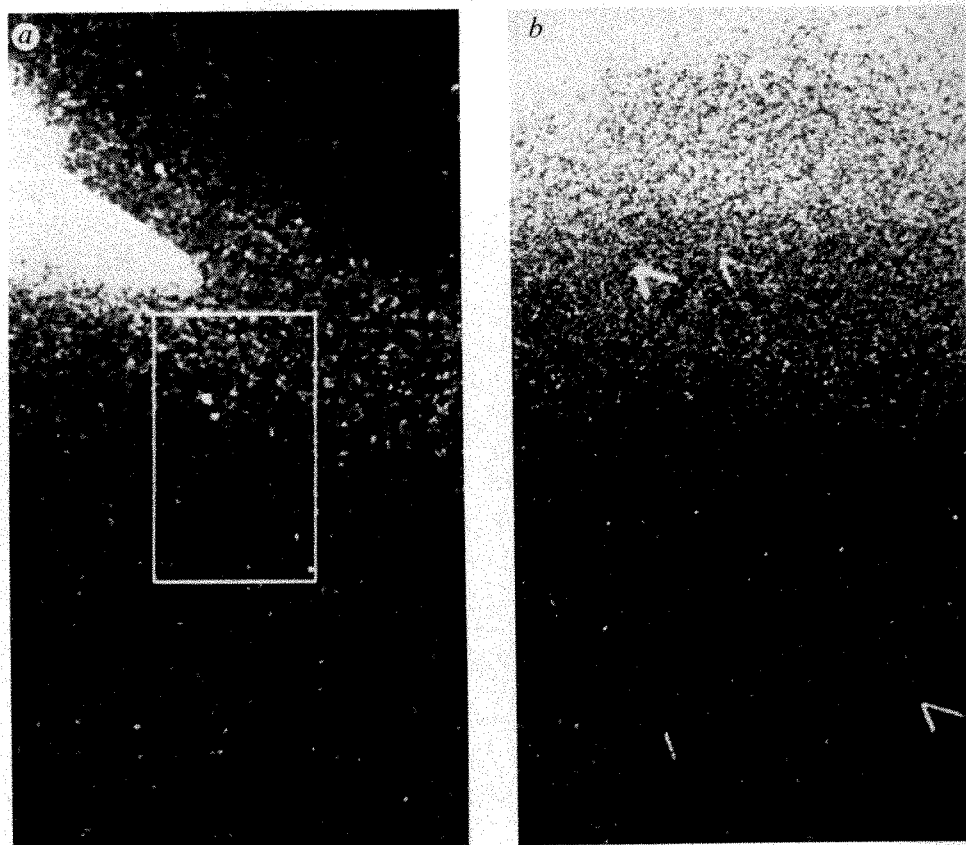
safely rule out the possibility that the stripe is a quirk of the Voyager vidicon.

This particular image has already received extensive scrutiny^{2,3} so it may seem surprising that the ring has been overlooked. Raw Voyager images receive extensive reprocessing on the ground, which includes the subtraction of a 'dark current', or image of dark sky, to model the null response of the vidicon. Previous versions of this frame were processed with an inappropriate dark current; this introduced a strong intensity gradient to the dark sky regions of the image, which masked this very subtle feature. We first noted the ring after subtracting an empirical fit to the background of the flawed image data, but found it easily once the image had been reprocessed with an updated dark current.

Only one additional Voyager image captures this ring with tolerable signal-to-noise ratio. The final frame (FDS 20693.01) of a narrow-angle (NA) mosaic accidentally fell beyond the bright ring's ansa, and so captured a piece of this faint structure (Fig. 2). However, the ring contributes only a smooth intensity gradient to the restricted NA field of view, which would have been impossible to interpret unequivocally without the context of the WA frame. Despite some uncertainty in this image's true dark current, it is compatible with the presence of the exterior ring⁴.

In retrospect, the additional ring was hinted at in earlier Pioneer 10 and 11 data. Pioneer 10's meteoroid impact detector recorded at least one event near ring plane crossing at 206,000 km (refs 5, 7), well outside the bright ring but within the ring described here. Since the instrument locks out additional detections for 80 min following an event, the actual number of impacts

Fig. 2 A comparison of the wide-angle (WA) and narrow-angle (NA) frames. *a*, A portion of the WA frame (FDS 20693.02), contrast-enhanced to show the gossamer ring. The rectangle outlines the corresponding NA field of view. *b*, The NA image (FDS 20693.01), using the same contrast enhancement.



during ring plane crossing is not known. Also, the Pioneer 11 charged particle absorption data, which first detected the bright ring⁶, have never been completely unravelled (W. Fillius, personal communication) but may require additional absorbers between Amalthea and the ring.

Since this 'gossamer' ring is only present at 0.5σ in the WA image, extracting its properties may only be accomplished by averaging over large numbers of the available pixels. Figure 3 is a radial profile of the ring, generated by averaging pixel intensities along contours of constant orbital radius. It shows that the ring decreases in intensity rather uniformly with radius; most of it lies inside the orbit of Amalthea, though some material extends out to 210,000 km, and perhaps to the neighbourhood of Thebe. Its overall mean brightness is $\sim 5\%$ that of the main ring. The profile's only notable feature is a $\sim 20\%$ enhancement centred at $160,000 \pm 2,000$ km, quite close to synchronous orbit (160,200 km). This bulge is significant at the 4σ level; in fact, it was first observed visually in the image, and only later found to fall on top of a special orbit. This unresolved feature is 5,000-km wide in the scan, but may actually be much narrower and brighter.

The profile of Fig. 3 is produced assuming that the ring is azimuthally symmetric, relatively thin and confined to the equatorial plane. A firm upper limit of 4,000 km may be placed on the ring's vertical extent from the width of the observed stripe at Voyager's shallow viewing angle (a mere 2° from the ring plane); although not severely constrained, this ring is distinctly thinner than the halo. For a ring ≥ 200 km thick, lines-of-sight from Voyager would sample a significant range of orbital radii through the ring plane. In this case, vertical structure would be confused with radial structure, and the profile would be in error. However, the synchronous feature must be < 200 km thick to be consistent with its width in the profile.

The ring shows some evidence for a variation in brightness with scattering angle θ over the limited range sampled. Intensity at constant orbital radius increases by nearly a factor of two between $\theta = 7^\circ$ and 6° , suggesting diffraction by grains of radius $r \approx 1.5 \mu\text{m}$ (Fig. 4). Although this strongly implies the presence of micrometre-sized grains, as in the main ring^{2,4}, it is not

possible to exclude the presence of either larger or smaller particles based on such a tiny range of θ (ref. 1). If the constituent particle size distribution is the same as that in the bright ring, then this ring's normal optical depth $\tau \approx 10^{-7}$, which is comparable to that of Saturn's E ring, another ring known for its small grain population¹. This value is compatible with $\tau \geq 10^{-8}$, determined from the Pioneer 10 impact detection near the ring's outer boundary, and applying only to particles larger than $r \approx 6 \mu\text{m}$.

We have failed to detect this ring in the three available backscattered views of the main ring's terminus (FDS 16368.19, 20630.52, 20630.53), despite averaging over many pixels; in these images, the exterior ring must be $< 1\%$ as bright as the main ring. This limit is smaller than the rings' forward-scatter intensity ratio of $\sim 5\%$, which suggests that the gossamer ring contains relatively fewer particles large enough ($r \gg 1 \mu\text{m}$) to backscatter efficiently⁴.

The dominant evolutionary process acting to move jovian ring material is plasma drag¹. This force arises from collisions with plasma, which is tied to the planet's rotating magnetic field; it evolves material away from synchronous orbit, either inward or outward, on timescales of $\sim 10^{3 \pm 1}$ yr for micrometre-sized grains. The dominant destruction process, sputtering, acts on similar timescales for these tiny grains¹.

Given such brief lifetimes, ring material must be continually replenished from some source for the ring to remain visible today. The alternative, that the ring is quite young, implies that our detection of it was an exceedingly unlikely event. The satellites Amalthea and Thebe, embedded near the ring's outer edge, are obvious candidates for source bodies; micrometeoroids impact their surfaces, injecting dust into the ring system¹. Due to its larger escape velocity, Amalthea is only $\sim 1/10$ as effective in this role as Adrastea, which contributes to the bright ring^{1,4}. However, Amalthea and Thebe's ejecta should evolve outwards under plasma drag, and so cannot fully explain the gossamer ring.

Unseen bodies provide a plausible alternative source, and these must extend across the synchronous orbit for the ring to also appear on both sides. Since sputtering and plasma drag act

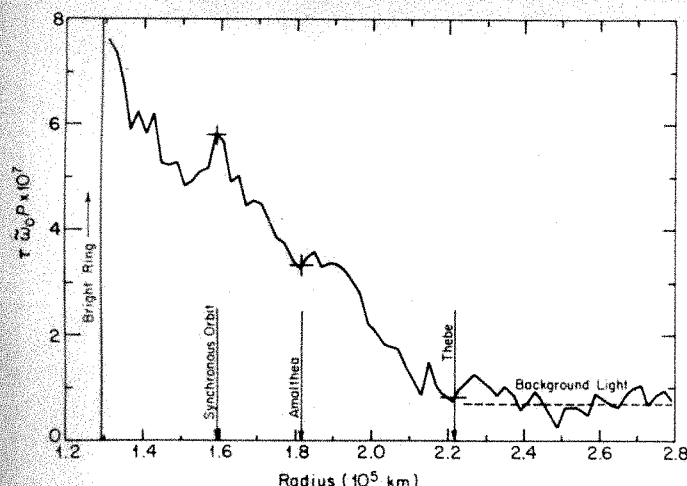


Fig. 3 A radial profile of the external jovian ring. The vertical axis is in non-dimensional units of $\tau \omega_0 P$, where τ is normal optical depth, ω_0 is single-scattering albedo and P is the phase function at scattering angle θ . This quantity is directly proportional to measured intensity, but eliminates various projection effects⁴.

on comparable timescales, we expect a steady decrease in brightness with distance from any source region. This may account for the ring's observed linear decay beyond synchronous orbit, implying that the source bodies are confined to a much narrower radial region than are the visible grains.

The bright feature at synchronous orbit appears surprising at first, since plasma drag drives material away from this location, not towards it. However, here the ring material and plasma corotate, so the plasma drag force vanishes. Hence, material injected into the ring at this orbit will evolve away more slowly (probably inward under the weak Poynting-Robertson drag force¹) than that introduced elsewhere. If the source body population straddles synchronous orbit, then this brightness enhancement is to be expected.

Another mysterious aspect of this ring is its limited vertical extent, <4,000 km. The halo is thickened by perturbations from Jupiter's inclined magnetic field, acting on its tiny, charged grains^{1,2,8}; the same processes should also be acting in this ring. The one important difference is the strength of the magnetic forcing. The dipole field component in the exterior ring is smaller by a factor of ~3, while higher-order moments are reduced even further. At the same time, grain velocities with respect to the

corotating field are smaller here, close to synchronous orbit. It is consistent with this trend that strong Lorentz resonances seem to have a major role in the halo⁸, but are not present in the gossamer ring. These circumstances combine to reduce the magnetic perturbations, and hence the ring's vertical extent, by a factor of at least 10 compared with the halo. This places the ring's thickness within its measured upper limit.

Although this gossamer ring remains enigmatic, it may at least have a dynamical counterpart at Saturn. A very faint outward extension to Saturn's A ring has been discovered by statistical analysis of the Voyager 2 photopolarimeter stellar occultation data⁹. Like its jovian analogue, it extends from a much brighter ring (Ring A) outwards to the vicinity of a satellite (Atlas), while decreasing continuously in optical depth. Its mean τ is ~2% of the outer A ring value. However, in its radial width (~900 km) and overall optical depth (~0.01), this ring is very different from the faint outer ring at Jupiter. Additional material at much lower optical depths, identified using image processing techniques similar to those described herein, fills the entire region between the A and F rings¹⁰. Whether these structures truly have a common dynamical basis is unknown, but surely searches should be made for similar material at Uranus.

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High time-resolution observations of periodic frictional heating associated with a Pc5 micropulsation

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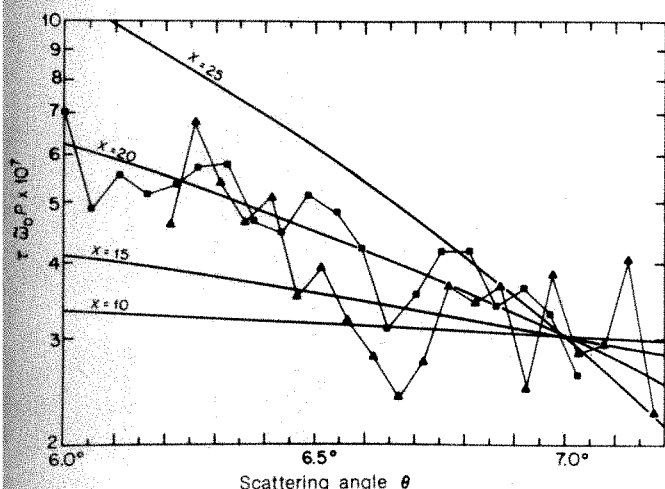


Fig. 4 Variations in ring intensity with scattering angle θ , measured at constant radius. Despite the point scatter, a brightening trend is revealed; a straight-line fit to the data yields a slope of $0.7 \pm 0.1 \text{ mag deg}^{-1}$. Phase functions for particles of size parameter X are shown for comparison⁴, where X is defined as the ratio of particle circumference to wavelength ($0.5 \mu\text{m}$). Δ , Near arm; $\blacksquare$, far arm.

Geomagnetic micropulsations are fluctuations of the Earth's magnetic field on time-scales ranging from a few seconds to several minutes and with amplitudes varying between a few and several hundred nanotesla. In the inner magnetosphere, where the Earth's magnetic field lines are closed, the oscillations can adopt a standing wave pattern with a scale size comparable to the dimensions of the magnetospheric cavity. These hydromagnetic waves constitute one of the primary mechanisms for the transfer of energy from the outer regions of the magnetosphere to the high-latitude ionosphere during geomagnetic substorms. In the ionosphere, Joule heating is thought to be the dominant damping process for the pulsations and the rate of energy dissipation during a typical event¹ is of the order of 10^9 W . By means of the EISCAT (European Incoherent Scatter) radar facility it has been possible to measure directly the ionospheric ion-temperature and ion-velocity fluctuations during a micropulsation. The relationship predicted by the theory of Joule heating for these two parameters is confirmed in detail by the high time-resolution results reported here.

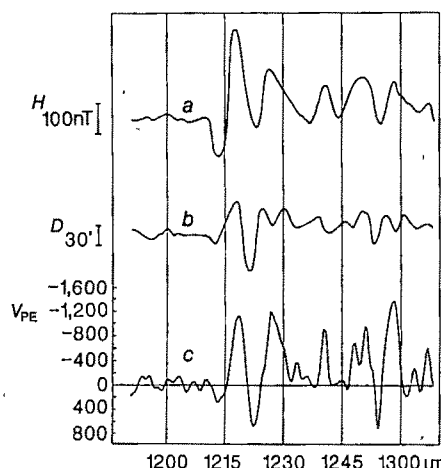


Fig. 1 Perturbations of *a*, the horizontal geomagnetic component, *H*, and *b*, declination, *D*, at Tromsø, Norway during a Pc5 micropulsation on 30 November 1982; *c*, ion velocity fluctuations derived from EISCAT data and resolved in the eastward-perpendicular direction.

Frictional heating in the thermosphere arises due to relative motion between the neutral and ionized particles. The electric field associated with a micropulsation drives the ions whilst the neutral motion remains essentially unchanged. Assuming that the neutral particles are stationary, theory predicts that Joule heating should produce an ion-temperature enhancement, ΔT_i , proportional to the square of the ion-velocity^{2,3}, v_i , according to

$$\Delta T_i = m v_i^2 / 3k \quad (1)$$

where m is the molecular mass of the neutral gas and k is Boltzmann's constant. This steady-state relationship is applicable to pulsation events since the time-scale of heating is much shorter than that of changes in the temperature and velocity of the neutral atmosphere.

On 30 November 1982, EISCAT was operating in the CP- ϕ mode, in which the Tromsø beam was directed along the Earth's magnetic field and the beams of the remote receiving antennas at Kiruna and Sodankylä intersected the Tromsø beam at an altitude of 312 km. Hence the full vector of plasma velocity in the common scattering volume could be determined⁴. At about 1215 UT a micropulsation occurred and large fluctuations in the ion-temperature and velocity were detected at Sodankylä. The ion-temperature fluctuations were ascribed to frictional heating^{5,6} but the time resolution of their data (200 s) was insufficient to establish a detailed correlation with the velocity perturbations. High time-resolution (30 s) data from all the EISCAT sites indicate that the ion-temperature fluctuations are precisely correlated with a rectified form of the ion-velocity perturbations, as predicted by theory (equation (1)).

The perturbations in the *H* (horizontal) and *D* (declination) components of the geomagnetic field at Tromsø, Norway, during the micropulsation are reproduced in Fig. 1*a* and *b*, respectively. The impulsive onset of the event coincides with a transient compression of the dayside magnetosphere at 1211 UT. This sudden commencement was not followed by a magnetic storm and the micropulsation was quickly damped. The pulsation period was ~ 9 min, hence the event is classified as a Pc5 micropulsation, although the wave amplitude was unusually large (~ 100 nT).

The ion velocity calculated from the EISCAT data and resolved perpendicular to the geomagnetic field also displayed large fluctuations with periods of 9 min during the pulsation (Fig. 1*c*), which were well correlated with the magnetic *H*-component variations. The amplitude of the eastward velocity component, V_{PE} , was 1 km s^{-1} , about 5 times that of the northward component.

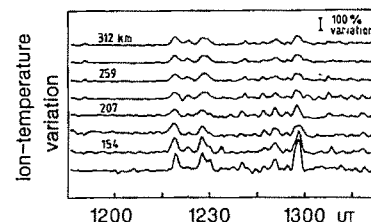


Fig. 2 Ion-temperature fluctuations scaled to the mean at each altitude, measured by EISCAT for altitudes of 130–312 km. The micropulsation event commenced at about 1215 UT.

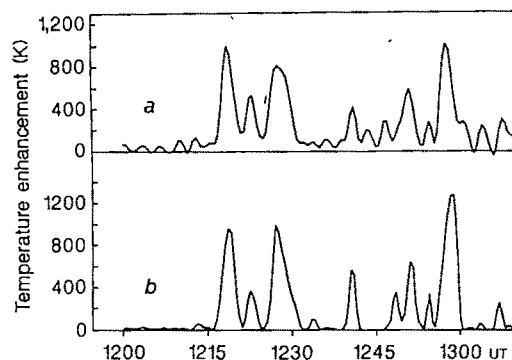


Fig. 3 *a*, Ion-temperature enhancements above the ambient value of 1,100 K, for an altitude of 312 km; *b*, ion-temperature perturbations derived from equation (1) in conjunction with ion velocities measured by EISCAT.

Transient enhancements of the ion temperature occurred at all heights in the range 130–312 km during the pulsation (Fig. 2). The high time resolution (30 s) of the data presented here permits the following interesting feature to be identified. The ion-temperature fluctuations are correlated precisely with a rectified form of the velocity perturbations during the micropulsation. This feature is illustrated in detail, for an altitude of 312 km, in Fig. 3 which compares the measured temperature variations (Fig. 3*a*) with theoretical predictions, using the measured velocities in conjunction with equation (1) (Fig. 3*b*). The ambient ion temperature at 312 km was $\sim 1,100$ K and the calculations reproduce temperature enhancements of between 400 and 1,000 K. Agreement between the two curves of Fig. 3 may be improved by taking the neutral wind velocity into account. However, this is likely to be $\sim 100 \text{ m s}^{-1}$, a fraction of the large ion velocities, and the correction is therefore small. The mean molecular mass employed in these calculations, $m = 17.1$ at 312 km, was derived from the US Standard Atmosphere, 1976. Similar excellent agreement between predicted and measured temperature perturbations was also obtained for other altitudes when appropriate molecular masses were employed in the calculation.

The ionospheric conductivity was approximately constant during the micropulsation⁵ and there is no correlation between the F-region electron and ion-temperature fluctuations during the event. Particle precipitation is not, therefore, responsible for the ion-temperature enhancements. On the contrary, the magnitudes of the measured temperature and velocity perturbations are quantitatively consistent with the expression given by equation (1) and these preliminary results thus provide indisputable evidence that large transient variations in ion temperature measured by the EISCAT radar during a Pc5 micropulsation are due to frictional heating. The results also support the hypothesis that ionospheric Joule heating is the primary damping mechanism for micropulsations.

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A 450-year drought reconstruction for Arkansas, United States

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A 450-year reconstruction of the June Palmer Drought Severity Index¹ (PDSI) has been derived for Arkansas using annual tree-ring chronologies from selected old-growth baldcypress trees (*Taxodium distichum* (L.) Rich.) found in floodplain swamps. This is the first palaeoclimate reconstruction achieved with baldcypress, and is some 150 years longer than any previous dendroclimatic estimate for eastern North America. The reconstruction is characterized by substantial fluctuation in the intensity and duration of past wet and dry spells, and is well verified against independent climate data. While there has been some very limited previous use of wet site species for dendrochronology^{2,3}, this is the first verified climate reconstruction based exclusively on a swamp-grown species. Several 500-800-year-long baldcypress chronologies are under development, and some may be extended into mid- to late-Holocene times with tree-ring records from preserved subfossil cypress logs⁴.

The extremely wet habitat of baldcypress contrasts sharply with the classic semi-arid tree-ring sites where dendroclimatology was first developed. Like many arid site trees, however, the radial growth of baldcypress and some other wet site species⁵ may be slow and highly variable, which may reflect, in part, the less than optimal excessive moisture conditions. The frequently inundated, alluvial nature of the cypress habitat also favours the rapid burial and long-term preservation of fallen cypress logs, a critical precondition for the development of millennia-long baldcypress tree-ring chronologies.

Baldcypress logging began in the late eighteenth century and reached a maximum around 1900 (ref. 6) but small relict stands of old-growth cypress remain scattered throughout its native range in the southeastern United States. We have discovered old, datable, and climate sensitive baldcypress on both blackwater and brownwater river systems⁷ (that is, streams characterized by organic and inorganic chemical loads, respectively), but have encountered dating difficulties with baldcypress from certain habitats near the southern limits of its range. Individual cypress >850-yr old have recently been sampled, and while older trees⁸ probably exist they have yet to be documented adequately. We have completed four baldcypress chronologies for Arkansas (Fig. 1), and include the three longest series in this analysis. These tree-ring sites have not been heavily disturbed and are not subject to high pollution levels, but all are located in drainage basins with extensive agricultural development.

Increment cores were extracted above the often pronounced root buttress, usually 2-4 m above the ground. The cores were properly mounted and surfaced, the annual rings were carefully cross-dated⁹ and measured, and the dating was independently confirmed. Non-climatic variance due to age trend and differential growth rates was minimized through a double-detrending

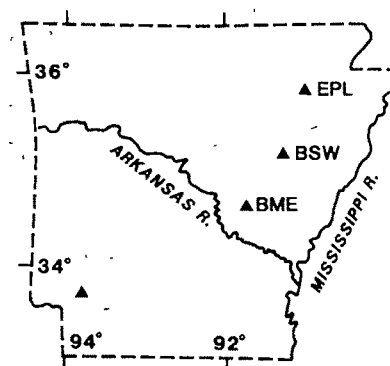


Fig. 1 Locations of baldcypress tree-ring chronologies in Arkansas. The three longest chronologies used to reconstruct June PDSI for Arkansas are: EPL, Egypt; BSW, Black Swamp; BME, Bayou Meto.

procedure, wherein negative exponential curves and stiff splines were fit in succession to the raw ring width data^{10,11}. The detrended, standardized ring width indices for all well correlated cores were averaged together by year into a robust mean value function chronology for each collection site (that is, the standard chronology). Autoregressive (AR) modelling of the tree-ring indices at each site revealed significant low-order autocorrelation in most series, believed to be due primarily to internal biological processes in baldcypress and to external ecological factors. To remove this suspected non-climatic growth persistence, a serially random residual chronology was also derived for each site¹². All ring width indices at each site were pre-whitened using second and third-order AR models selected on the basis of the Akaike final-prediction-error criterion¹², and the resulting serially-random residuals were then averaged yearly into the robust mean residual chronology for each collection site¹⁰. A small amount of variance trend remained in the standard and residual chronologies, and was stabilized by fitting a very inflexible spline to the variance¹⁰.

Direct³ and inverse¹³ correlations between growing season rainfall and the growth of certain wet-site species have been reported. Correlation and response function analyses¹⁴ of our available cypress chronologies both indicate that baldcypress radial growth is correlated directly with precipitation and inversely with temperature for several months during the current growing season, in spite of frequently flooded site conditions. Using the appropriate divisional climate data for 1931-80, these correlations range as high as +0.58 for June precipitation and -0.43 for June temperature.

The response of internal baldcypress growth regulating mechanisms to environmental conditions is not well understood, but we emphasize that baldcypress is physiologically adapted to wetland conditions and remains subject to the environmental variation inherent in its swamp habitat, as documented in part by the climatic correlations reported above. We suspect that complex factors related to and including rainfall amounts may limit the growth of baldcypress. These conditions probably include periodic drydown of the root system during drought, moderation of direct solar radiation by cloud cover, high temperatures, and lower water quality during drought. High temperatures and intense solar radiation, for example, might cause transpiration rates to exceed moisture uptake and would result in internal moisture stress even in saturated soil conditions. Field monitoring of baldcypress conditions, and improved modelling of the growth-climate relationship will be required for a better definition of the environmental response of baldcypress. Nevertheless, the observed correlations with precipitation and temperature imply that useful estimates of past summer drought can now be derived with these long baldcypress chronologies.

Statewide average PDSI has been calculated monthly from 1891 to 1982 for Arkansas¹⁵. It is based on areally-weighted divisional temperature and precipitation values from 1931 to

1982, and on adjusted equally-weighted values from all available reporting stations for 1891 to 1930. Although the number of reporting stations declines and missing values increase before 1931, records for 29 stations extend to 1891 and provide reasonably uniform spatial coverage of the state¹⁶.

Equations for calibrating the tree-ring and PDSI series to predict past drought were derived for the standard and residual chronologies using both simple and stepwise multiple regression. Calibrations were restricted to the period 1931–80 when the PDSI data are of the highest quality. Several monthly and seasonal PDSI values for the current growing season were examined as dependent variables (that is, April, May, June, July, May–June, May–July, April–July), and three tree-ring chronologies were used as independent variables.

Independent PDSI data for 1891–1930 not included in developing the calibration equations were used to verify and compare each drought reconstruction. The correlation coefficient, sign test, and the reduction of error statistic were used for verification^{14,17,18}. These tests indicate that the best results were obtained using simple linear regression between statewide June PDSI and a regional average of the three mean residual tree-ring chronologies (Fig. 2). The regression equation used for calibration is:

$$y = -5.189 + 5.102x$$

where y is the estimated June PDSI for a given year and x is the regional average of the residual chronologies for the same year. This model is significantly correlated with June PDSI ($r = 0.64$, $P < 0.0001$) during the calibration period, and explains 40% of the variance in the PDSI series after adjustment for loss of degrees of freedom (r_{adj}^2).

The verification statistics between observed and estimated June PDSI for 1891 to 1930 indicate that the reconstructed series is a stable, reliable indicator of past drought. The correlation between the observed and estimated drought series is 0.70 ($P < 0.0001$) during the 40-yr verification period, which explains some 49% of the variance in the independent climate series. The sign test indicates 29 agreements and 11 disagreements ($P < 0.01$) during the verification period. And the reduction of error (RE) for the verification period is +0.42, indicating that the model accurately reconstructs actual drought conditions. (Significance tests are not available for the RE statistic, which ranges from $-\infty$ to +1.0, but simulation experiments suggest that the approximate 95% confidence level is $RE \approx 0.0$ for $n > 10$ (ref. 18).)

Acceptable verification statistics were obtained when the standard chronologies were used in simple and stepwise multiple regression models with June PDSI, but the statistics indicate that the regional average of the residual chronologies provide the most accurate climate reconstruction (explaining some 7% more climate variance, with an RE statistic 0.14 points higher during the verification period). Removing low-order persistence from the residual chronologies has evidently enhanced the climate signal in these chronologies, implying that the persistence is largely physiological in nature and is not in phase with possible climate persistence.

The generally good agreement between the annual and multi-year departures for the observed and estimated drought series in both the calibration and verification periods is apparent in Fig. 2. Careful examination of Fig. 2 and the actual climate data suggests that the reconstruction may be even more reliable than indicated by the verification statistics. The three worst estimates during the verification period were calculated for 1895, 1928, and 1902, the three years by rank with the largest disparity between the observed and estimated June PDSI (Fig. 2). In each of these years, however, June rainfall was well above average and reversed the climate regime established in the preceding months¹⁵. Moderate June drought was reconstructed for 1895 and 1902, but heavy June rains fell in both years and broke 13 consecutive months of incipient to moderate drought in 1895, and 16 consecutive months of mild to severe drought in 1902. Normal moisture conditions were reconstructed for June of 1928, but the highest average June rainfall in 92 yr was actually

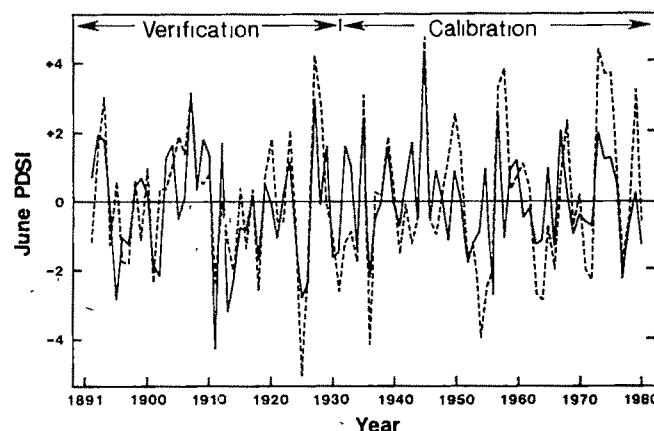


Fig. 2 Actual (dashed line) and reconstructed (solid line) statewide June PDSI for Arkansas, 1891–1980. The reconstruction was calibrated on the 1931–80 data, and verified with the pre-1931 data.

recorded for Arkansas in 1928, which produced a moderate moisture excess ($PDSI = +2.85$) and reversed five months of slightly below average rainfall¹⁵.

These examples illustrate differences in the way the Palmer Index and baldcypress trees integrate the effects of current and previous climate. Baldcypress growth is generally not well correlated with drought conditions during the preceding winter and early spring. But as the 1895, 1902, and 1928 data suggest, particularly severe or prolonged dry spells during this period can result in reduced radial growth. Improved conditions in June may be reflected by the Palmer Index, but apparently come too late on these occasions to reverse the established growing conditions for baldcypress.

If 1895, 1902, and 1928 are excluded from the verification period, the simple correlation increases to 0.79 ($P < 0.0001$) and the RE increases to +0.59. These verification tests provide convincing evidence regarding the overall accuracy and reliability of the long-term drought reconstruction.

Detrending, AR modelling, and regional averaging of chronologies from three relatively undisturbed sites has minimized but probably not entirely eliminated possible non-climatic trends from the reconstruction due to natural or artificial disturbances (such as, insect defoliation, selective logging, and land clearing). For example, the estimated series does not fully reflect the extremes in the actual data, especially after about 1950 (Fig. 2). The variance of the actual and estimated PDSI series for 1891 to 1949 is 3.55 and 2.85 respectively, but changes to 5.44 and 1.69 for 1950 to 1980. The drop in reconstructed variance after 1950 reflects reduced variance in the tree-ring chronologies. In view of this unexplained variance, the 450-yr drought reconstruction should only be considered as a conservative estimate of past moisture anomalies, and probably does not fully reflect the extremes of wetness and dryness which actually occurred.

If the drop in variance after 1950 is a real nonrandom phenomenon, it may be related to increased post-war developments in the lower Mississippi Valley. The effects of various artificial developments on baldcypress growth are likely to be complex, but greatly accelerated land clearing and land draining for agriculture in recent decades¹⁹ have changed water levels, the nutrient budget, and other ecological aspects of affected wetlands⁷ and may be among the most prominent impacts in the lower Mississippi Valley. The apparent loss of variance after 1950 needs further documentation with additional baldcypress chronologies, but suggests that the reconstruction might be improved by basing the calibration on pre-1950 data.

Reconstructed June PDSI for 1531–1980 is plotted along with a filtered version designed to pass variance with a frequency of ≥ 8 yr (Fig. 3)¹⁴. Numerous wet and dry spells comparable with twentieth century events punctuate the 450-yr reconstruction.

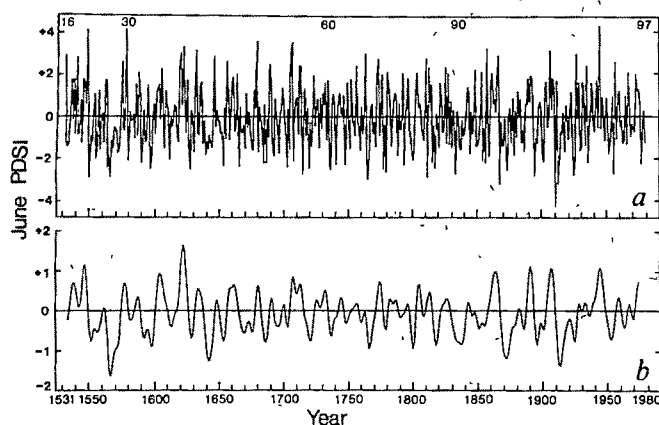


Fig. 3 Reconstructed statewide June PDSI for Arkansas, 1531-1980 (a) and a low-pass filtered version (b). Sample size decreases from 97 cores at 1980 to 16 at 1531, as noted above the upper plot.

Ten droughts ≥ 10 yrs in length are estimated in the filtered series for around 1555, 1570, 1595, 1670, 1765, 1835, 1850, 1875, 1900, and 1915 (Fig. 3b). Five of these droughts have occurred in the past 150 yr, including what may have been two of the three most intense and prolonged dry spells in the entire reconstruction (that is, 1875 and 1915). The prolonged droughts estimated for around 1550 and 1570 are also obvious in the filtered plot, and reflect negative PDSI values for 22 of the 29 yrs between 1549 and 1577 in the unfiltered series (Fig. 3a). This may represent the worst June drought period for Arkansas in the past 450 yr.

Another striking feature of the filtered reconstruction is the apparent increased amplitude of moisture anomalies before 1650 and after ~ 1840 (Fig. 3b). In view of the variance stabilization and good sample size throughout the reconstruction (Fig. 3), we are inclined to attribute these changes to real macroenvironmental factors.

This research demonstrates the suitability of swampgrown baldcypress for palaeoclimatic reconstruction in the southeastern United States. Some climate reconstructions based on upland species have produced better verification statistics²⁰, but very few species other than baldcypress have definite potential to provide millennia-long tree-ring chronologies in eastern North America. The additional baldcypress chronologies currently under development throughout the Atlantic and Gulf coastal plains should result in significantly longer drought histories, and should permit investigation of the spatial distribution of past moisture anomalies in the south-east.

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Dioxin radical formation and polymerization on Cu(II)-smectite

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Decontamination of hazardous wastes containing dioxins (dibenzo-*p*-dioxin and its chlorinated analogues) remains a serious problem and new approaches for the degradation or alteration of dioxins are needed. We describe here dibenzo-*p*-dioxin radical cation formation and polymerization on a simple clay mineral (Cu(II)-smectite) in mild reaction conditions. Existence of the radical species was conclusively demonstrated using electron spin resonance (ESR), ultraviolet-visible, and infrared spectroscopy. The radical cation polymerized to form dimers and trimers as revealed by mass spectroscopy. Radical cations of 1- and 2-chlorodibenzo-*p*-dioxin were also formed on Cu(II)-smectite. The radical cations are formed by electron transfer from the dioxins to Cu(II). The formation of dioxin radicals may provide the basis for new detoxification methods in which the reactive radical species is coupled to other molecules or degraded by subsequent reactions.

One of the principle unsolved problems concerning dioxins is the development of effective and economically feasible methods to detoxify them. Large quantities of dioxin-contaminated manufacturing wastes remain buried at various sites in the US. There are also $\sim 3,000$ barrels of dioxin-contaminated wastes stored in an Environmental Protection Agency (EPA) approved shelter¹. The most widely used method for detoxification of dioxin is incineration. Other approaches including concentration, photolysis, radiolysis, ozonolysis, chloriodide degradation, wet-air oxidation, chlorinolysis, chlorolysis, catalytic dechlorination, destruction by ruthenium tetroxide and biological treatment have been described but not widely used¹⁻³.

Smectites are layer silicate clay minerals possessing a net negative charge which is compensated by exchangeable cations. Several aromatic molecules have been shown to form radical cations when adsorbed by Cu(II), Fe(III) or VO(II)-smectite⁴⁻¹⁰. This results from complete electron donation from the aromatic species to the metal cation with attendant reduction of the metal ion. Such reactions have been followed by means of infrared and ESR spectroscopy. The radical cations once formed, may then undergo polymerization as shown for benzene which forms *p*-polyphenyl polymers of various molecular weights¹⁰ and for anisole (methoxybenzene) which forms 4,4'-dimethoxybiphenyl⁸. The development of such radical cations in relatively mild reaction conditions suggests unique effects of the negatively charged silicate surface in promoting their formation. The objective of the present study was to determine if dibenzo-*p*-dioxin, 1- and 2-chlorodioxins would undergo similar radical cation formation and polymerization reactions on smectite surfaces. Earlier work has shown the existence of dioxin radical cations in homogeneous solutions of concentrated sulphuric acid with oxidizing agents such as H₂O₂ and KNO₃ (refs 11, 12) and in homogeneous solutions of trifluoromethanesulphonic acid exposed to ultraviolet light and O₂ (ref. 13).

When Cu(II)-smectite was refluxed (69 °C) in a solution of *n*-hexane in which dibenzo-*p*-dioxin (referred to as dioxin in the following discussion) had been dissolved, the normal light blue colour of the clay was replaced by a deep blue colour suggestive of the dioxin radical reported to be formed in

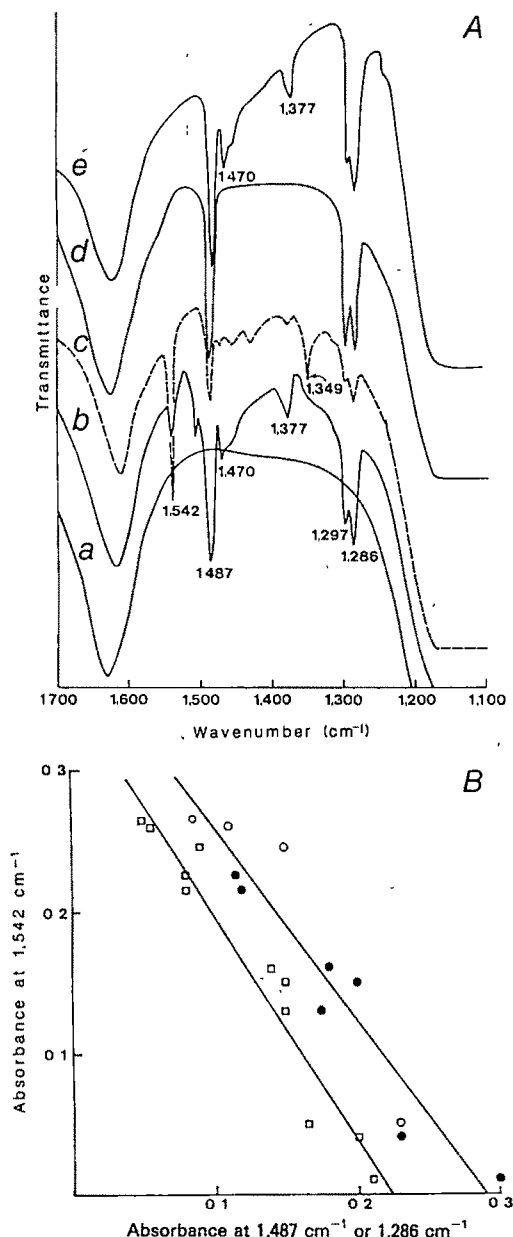


Fig. 1 A, *a*, Infrared spectrum of a film of Cu(II)-smectite; *b*, same film after refluxing for 2 h in hexane containing dissolved dibenzo-*p*-dioxin; *c*, after evacuation in a cell for 1 h; *d*, re-exposed to the atmosphere for 2 weeks; *e*, Ca²⁺-smectite refluxed 2 h in hexane containing dissolved dioxin. The clay films were obtained by evaporating a water suspension of the clay on sheets of polyethylene. After drying, the clay films ($\sim 1 \text{ mg cm}^{-2}$) could be peeled away from the plastic. After refluxing, the self-supporting films were placed in an evacuable infrared cell fitted with AgCl windows, and the infrared beam passed directly through the film. B, Plots of the absorbances of two bands, 1,487 cm⁻¹ (circles) and 1,286 cm⁻¹ (squares) attributed to the molecular form of dibenzo-*p*-dioxin versus a band attributed to the radical cation (1,542 cm⁻¹). The various points represent a variety of proportions of the two configurations produced by different levels of hydration of the clay films. O, Proportions of the two species as the film was dehydrated; ■, as the film was rehydrated.

homogenous solution¹¹⁻¹³. As in the work reported for other aromatic species on Cu(II)-smectite, the refluxing process probably dehydrates Cu(II) to the point where the dioxin molecule can interact with the Cu(II) through π electrons at vacant ligand positions. At this point an electron is completely transferred reducing the Cu(II) to Cu(I) and producing the radical cation of dioxin. Evidence for this reaction is from the following: infrared, ultraviolet-visible, ESR, and mass spectroscopy.

Figure 1 shows the infrared spectra of a Cu(II)-smectite film

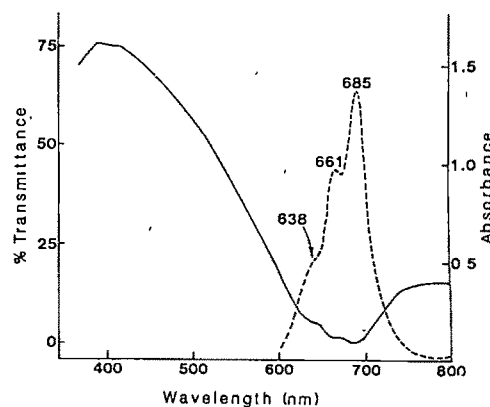


Fig. 2 Ultraviolet-visible differential spectra of the dioxin radical on Cu(II)-smectite recorded in both absorbance (dashed line) and transmittance (solid line) modes. An aqueous suspension of Cu(II)-smectite was placed on the inside surface of both the sample and reference cells and evaporated at room temperature forming a continuous clay-film. The sample cell was then placed in a round bottom flask and refluxed in hexane which contained dioxin. Within an hour the film turned a deep blue colour indicative of the radical. The sample cell was removed from the flask, filled with hexane, and placed in the sample beam. Additional hexane was used to fill the reference cell. Spectra were recorded on a Perkin Elmer 320 spectrophotometer.

(*a*) and the same film (*b*) after refluxing in a hexane solution of dioxin, and placed in a vacuum cell. Prolonged evacuation of the cell produces maximum development of the bright blue colour (*c*), and then exposure to moist air reconverts the colour to the light blue typical of Cu(II)-smectite (*d*). Figure 1*e* shows the spectrum of dioxin on Ca²⁺-smectite. Bands unique to the molecular form of dioxin are at 1,286, 1,297, 1,377, 1,470, and 1,487 cm⁻¹. Those which form with the development of the bright colour and are, therefore, related to the radical cation are at 1,349 and 1,542 cm⁻¹. Assignment of the bands has not been made, but are probably ring stretching and deformation vibrations. Figure 1 also shows the inverse relationship of the absorbance of two molecular bands, 1,487 and 1,286 cm⁻¹, with the radical cation band at 1,542 cm⁻¹ (correlation coefficient, $r > 0.95$). These data show that as the radical cation is formed, the amount of molecular species decreases, then as moist air is admitted the band of the radical species decreases and those of the molecular species increase. Figure 1*d* shows the spectrum of the same film after standing in air and may represent mainly the dimer of dioxin which has formed by radical cation interaction with molecular forms. The molecular bands of dioxin at 1,377 and at 1,470 cm⁻¹ are missing in this material, while those at 1,286, 1,297, and 1,487 cm⁻¹ are maintained. No colour change or radical bands at 1,349 and 1,542 cm⁻¹ appeared in the Ca-system (Fig. 1*e*) even after prolonged refluxing and evacuation.

The visible spectrum of the bright blue complex is given in Fig. 2. A broad absorption band in the red region is characterized by a maximum at 685 nm and two lesser peaks at 661 and 638 nm, which are particularly well expressed in the absorbance mode. Figure 2 represents a differential spectrum since the reference beam passed through a Cu(II)-smectite film in hexane, while the sample beam passed through the Cu(II)-smectite-dioxin complex in hexane. The position of the main peak (685 nm) is not far from that of the dioxin radical (655 nm) developed in homogeneous solution¹³. This difference could easily be accounted for by the interaction of the dioxin structure with the silicate surface, which could result in minor band shifts.

The ESR spectrum of the Cu(II)-smectite-dioxin complex (Fig. 3*a*) exhibited a narrow line ($H_{pp} = 1.5 \text{ G}$) at $g = 2.0028$ conclusively demonstrating the existence of the dioxin radical cation. The Cu(II) ESR signals apparent in Fig. 3*c, d* were not observed in the dioxin spectrum demonstrating that paramagnetic Cu(II) was reduced to diamagnetic Cu(I) by electron transfer from dioxin to Cu(II). No hyperfine structure was

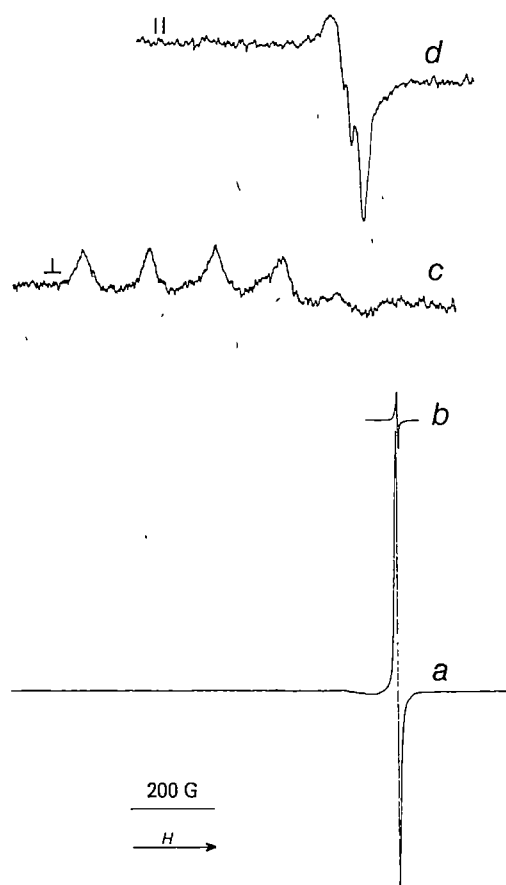
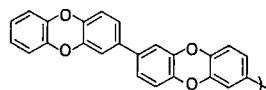


Fig. 3 ESR spectra of: dioxin radical cations on: *a*, Cu(II)-smectite film; *b*, standard pitch ($g = 2.0028$) Cu(II)-smectite film with H_0 oriented perpendicular (*c*) and parallel (*d*) to the clay layers. The radical cation was formed by refluxing dioxin and a mixed Cu(II)-Ca²⁺-smectite film (1:9 molar ratios of Cu:Ca) in hexane for 2 h. The film was then placed in a quartz tube which was evacuated for 2–3 h. The evacuated tube was then placed into a Varian E-4 spectrometer and the spectrum recorded. Spectra *c* and *d* were recorded in a similar fashion using mixed Cu(II)-Ca²⁺-smectite films that were not exposed to dioxin. Orientation of the clay films with respect to H_0 separated the $g_{\perp}$ and $g_{\parallel}$ components of the Cu(II) signal.

observed for the dioxin radical on Cu(II)-smectite. Proton hyperfine splitting has been observed for dioxin radical cations in homogenous solution¹³; the loss of hyperfine structure has been attributed to rapid electron exchange on the smectite surface and is consistent with ESR spectra reported previously for other aromatic molecules which form radical cations on transition metal layer silicates⁹.

Evidence for the formation of dioxin polymers on Cu(II)-smectite was obtained using mass spectrometry (Fig. 4). Molecular ions were observed for dioxin dimers (m/e 366) and



trimers (m/e 548) of the type shown in Fig. 5. The dioxin monomer (m/e 184), dimer and trimer were chromatographed separately with increasing temperature. Polymerized products of benzene, phenol, and anisole on Cu(II)-smectite have been observed previously^{8,10}.

The reaction described above for dibenzo-*p*-dioxin has also been found to proceed for 1- and 2-chlorodibenzo-*p*-dioxin as shown by brilliant blue colour development when the materials were refluxed in hexane with the mineral. Infrared absorption studies on these clay films showed development of new bands correlated with the colour formation. Thus, the radical cation

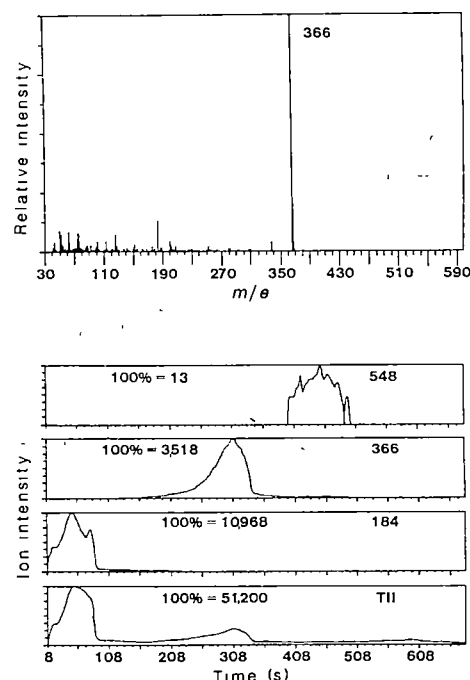


Fig. 4 Mass spectrum and mass chromatographs of a methanol extract of the Cu(II)-smectite-dioxin complex. The complex was formed by refluxing Cu(II)-smectite and dioxin (1:2 molar ratio of Cu:dioxin) in hexane for 6 h. The hexane was decanted and the solid material extracted with 15 cm³ of absolute methanol. The methanol extract was then decanted and evaporated to dryness under a stream of air. A small portion of the solid material obtained was introduced into a Hewlett-Packard 5985A mass spectrometer using a solid probe. The sample was held at 30 °C for 1.5 min then heated 30 °C min⁻¹ to a maximum temperature of 280 °C. Mass chromatographs are shown for the total ion intensity (TII) and m/e values of 184 (dioxin monomer), 366 (dioxin dimer) and 548 (dioxin trimer). The mass spectrum displayed was taken at 5.2 min.

formation is possible with chlorinated members of this group. When dibenzo-*p*-dioxin was refluxed in hexane with Fe(III)-smectite, radical cation formation was also noted, as it was observed in other work on aromatic radical cation formation in clays⁹. This was suggested by the development of a dark green colour in the clay, probably resulting from the combination of the blue of the radical cation and the colour bestowed by various valence states of iron.

Our work suggests possibilities for detoxification of dioxins (including the chlorinated congeners) through catalysis by a simple mineral material. The relatively mild reaction conditions plus the availability of a cheap and easily-prepared clay material seems attractive. Additional research may reveal the possibilities of reacting the radical cations of dioxins in the clay with other organic species to yield different kinds of reaction products which might be more desirable than polymers of the dioxin itself. The reactive radical species may also be subject to other kinds of reactions leading to the formation of less toxic products.

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Hydroxymethyl hydroperoxide and bis(hydroxymethyl) peroxide from gas-phase ozonolysis of naturally occurring alkenes

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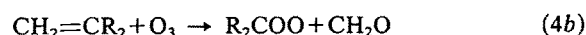
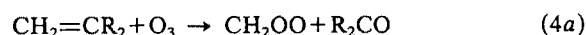
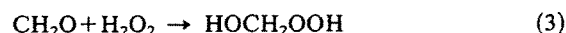
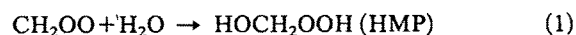
Ozonolysis is one of the main pathways for degradation of alkenes in the atmosphere, where the reaction is associated with smog formation¹ and the haze that often occurs over forests^{2,3}. In recent decades, ground-level ozone concentrations have greatly increased^{4,5}. The possibility of significant damage to plants by ozone and its products has consequently been raised in discussions of 'waldsterben', the large-scale dying of trees in northern Europe and North America⁶⁻⁹. With particular regard to the formation of peroxides, we have used ¹³C-nuclear magnetic resonance (NMR) to investigate the water-soluble products of the gas-phase ozonolysis of isoprene and several terpenes, which are emitted into the atmosphere in large quantities (10⁸–10⁹ tonnes yr⁻¹ globally) by trees^{3,10,11}. All these alkenes yield bis(hydroxymethyl)peroxide (BHMP; HOCH₂OOCH₂OH). The apparent precursor of BHMP is hydroxymethyl hydroperoxide (HMP; HOCH₂OOH), which results from addition of water to the ozonolysis intermediate. As both HMP and BHMP have various toxic effects on plant cells and enzymes¹²⁻¹⁴, we point out here an indirect way by which ozone may adversely affect forests.

Although it is a powerful tool for the determination of the structures of organic compounds, NMR has until now been neglected in favour of Fourier transform infrared spectroscopy (FTIR) and mass spectrometry (MS) in studies of gas-phase ozonolysis, because it requires larger samples and transfer of the products to solution after the reaction. Our experiments were carried out with alkene and O₃ concentrations much higher than those found naturally. In a typical experiment, alkene (2.65 mmol), H₂O (9.45 mmol; calculated to give ~50% relative humidity at atmospheric pressure) and air were introduced into an evacuated 20-l pyrex vessel to a total pressure of 100 torr, followed by ozone (6–7 mmol; ~5% in O₂) and air until atmospheric pressure was reached. An aerosol began to form immediately and after 1 h had largely settled out onto the walls of the reactor. The vessel was washed with a solution of 2 ml of D₂O in 200 ml of acetone; after removal of the acetone under vacuum, dioxane (10 mg) was added as an internal standard. The ¹³C-NMR spectra were recorded at 5–10 °C, with no pulse delay other than the 0.8 s acquisition time. The BHMP yields from some representative experiments are shown in Table 1; the reported yields have been corrected for some loss of BHMP during acetone-removal.

Isoprene and α-pinene are the main alkene components of forest air, where they occur in concentrations as high as 1–10 p.p.b., parts per 10⁹ (refs 3, 7). We have examined the ozonolysis of these compounds, 2-chloropropene, and the naturally-occurring alkenes ethene, β-pinene, 2-carene and limonene. In every reaction where O₃/alkene was ≥1, we found BHMP (δ=94.4) and formic acid (δ=168.5). In most reactions, lesser amounts of HMP (δ=95.4) and formaldehyde (δ=84.6) were also found. Although the resonances of BHMP and HMP lie close together, they were shown by admixture of the authentic

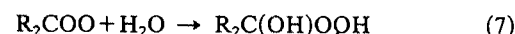
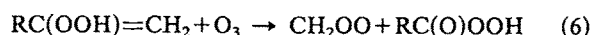
substances to be reliably separated from each other and from 1,3,5-trioxane (formaldehyde trimer) (δ=96.2) and 1,2,4-trioxolane (ethene ozonide) (δ=95.1). (Ethene ozonide is a possible product of the reaction, but would almost certainly be removed from the sample along with acetone).

Equations (1) and (2) show the pathways by which HMP and BHMP are probably formed. The alternative route to HMP in equation (3) is possible, but not necessary, as will be shown below. Reactions (3) and (2) represent the known syntheses of HMP and BHMP^{15,16}; reaction (1) has been postulated for gas-phase ozonolysis¹⁷ and is a typical reaction in aqueous solution¹⁸.



The reactants in equations (1)–(3) are all known products of ozonolysis. According to the Criegee mechanism, both CH₂OO (carbonyl oxide) and formaldehyde can be produced in the reaction of ozone with terminal alkenes [equations (4a, b)]¹⁸. In the gas phase, particularly at low pressures, most of the excited CH₂OO is lost by unimolecular reactions leading to various free-radical and molecular products including water. At pressures near atmospheric, a significant fraction of this species is collisionally stabilized, and bimolecular reactions [see, for example, reaction (1)] become more important^{19,20}.

Alkenes without the group CH₂= can also form CH₂OO and CH₂O by a route which has been suggested for both solution²¹ and gas-phase ozonolyses²². Thus, as shown in equation (5), a carbonyl oxide with a methyl substituent can stabilize itself by an internal hydrogen shift to produce a new, terminal alkene. Depending on the relative reactivities of the original and the new alkenes and the amount of ozone available, the new alkene may also be cleaved. The electronegative hydroperoxy group, however, will make the second ozonolysis highly selective for CH₂OO [equation (6)]¹⁸.



Ozonolysis in aqueous solvents produces hydrogen peroxide¹⁸, presumably by addition of water to the carbonyl oxide, followed by elimination, as in equations (7) and (8). Because of its chlorine substituent, 2-chloropropene will selectively yield CH₂OO and acetyl chloride. Here processes involving H₂O₂, as in equations (3), (7) and (8), cannot play a significant role, because addition of H₂O to CH₂OO delivers HOCH₂OOH (HMP) directly. Ozonolysis of 2-chloropropene gives a good yield of HOCH₂OOCH₂OH (BHMP), indicating that the formaldehyde needed to produce BHMP is formed under our conditions either from the reaction of CH₂OO and ozone²² or from radical reactions of the alkene and its degradation products.

Ethene and isoprene are expected to yield both CH₂O and CH₂OO, and we find that they give significant amounts of BHMP even when ozone/alkene < 1. β-Pinene also has the structural element CH₂= and, as expected, it yields a considerable amount of BHMP. Within the rather large measurement errors of ¹³C-NMR, the yield decreases only slowly as O₃/alkene drops below 1.

The case of α-pinene, which has no terminal double bond, is quite different. Here the yield of BHMP is sharply reduced as the ratio of O₃ to α-pinene decreases (Table 1, experiments 1, 2 and 3). This is consistent with the hypothesis that CH₂OO from α-pinene results from ozonolysis of the rearrangement product of the initial carbonyl oxide [equations (5) and (6)].

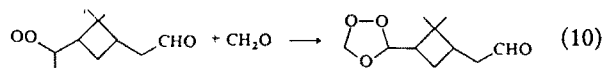
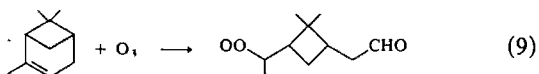
Table 1 Yield of BHMP from gas-phase ozonolysis

Experiment*	Alkene	Alkene (mmol)	O ₃ Alkene	BHMP (mmol)
1	α -Pinene	2.65	2.60	0.28
2		2.65	1.46	0.07
3		2.65	0.72	<0.01
4		2.65	0.85	0.06†
5	β -Pinene	2.65	2.34	0.16
6		2.65	0.84	0.10
7	Limonene	2.65	2.73	0.18
8	2-Carene	2.65	2.83	0.20
9	Isoprene	2.65	2.58	0.66
10		2.65	0.87	0.18
11	Ethene	6.67	1.06	0.64
12	2-Chloropropene	4.71	1.81	0.48

* All experiments except 12 were for 1 h at room temperature; experiment 12 was for 17 h at 5 °C.

† 3 mmol CH₂O were added to the reactor before ozonolysis.

Published data on ozonolysis of α -pinene are inconsistent about the direction of cleavage of the (presumed) primary ozonide^{3,23,24}. We find, however, that good yields of the cross ozonide shown in equation (10) can be obtained by ozonolysis of α -pinene in solution in the presence of CH₂O; this is clear evidence of cleavage as in equation (9) (to be published elsewhere). The presence of CH₂O in the α -pinene reaction can be shown, nevertheless, even with O₃/ α -pinene <1 (see Table 1, experiment 4, where formaldehyde is added), suggesting that the rate of ozonolysis of the rearranged carbonyl oxide is comparable with that of α -pinene.



Formation of BHMP in the ozonolysis of simple alkenes, for example, ethene^{25,26}, has been known for a long time. HMP was never detected among the ozonolysis products, probably because it is a liquid and less stable than BHMP. An ion at m/e 64, corresponding to the molecular ion of HMP, has been observed in the gas-phase ozonolysis of ethene and 2-methylpropene, but the investigators were unable to explain to their satisfaction how HMP could be formed in their low-pressure system¹⁷. They did, however, recognize that the addition of water would be an important loss process for carbonyl oxides in the atmosphere²⁷. The direct identification here of HMP and BHMP confirms these reports, but we emphasize that the reaction of water with CH₂OO is a possible environmental source of the peroxide HMP at elevated ground-level O₃ concentrations.

The high alkene and O₃ concentrations used here probably do not affect the sequence of reactions leading to HMP from isoprene and terpenes with terminal double bonds. In view of the huge annual emission of terpenes by plants and the availability of O₃ and water vapour, it is possible that considerable amounts of HMP are formed in the environment. The low concentrations of formaldehyde in the atmosphere make it less likely that much BHMP is formed. Similar considerations also apply to the question of whether HMP and BHMP are formed in smoggy urban atmospheres, where both ozone and alkenes (for example, ethene and propene) are known to be present¹.

There have been several investigations of the biological activities of BHMP and HMP. BHMP has been shown to be a mutagen in experiments on root meristem cells (*Vicia faba*)¹², as well as in *Drosophila*²⁸ and *Neurospora*²⁹, and it has cytostatic activity in mouse tumour cells³⁰. Several enzymes, especially those with —SH groups, are inhibited by BHMP^{13,31}; it may be significant that horseradish peroxidase is irreversibly inhibited¹⁴.

Two studies on BHMP have indicated that the effects observed resulted from a product, presumably HMP, formed by hydrolysis of BHMP in the test system^{12,13}; the possibility that these were the results of further hydrolysis of H₂O₂ could be eliminated^{29,32}. Despite the implications of these results, there appear to have been no studies of the response of whole plants or mammals to HMP and BHMP. Both of these substances are rather unstable under physiological conditions³³, and it is unknown whether they can enter intact plants. It is important that attempts be made to detect HMP in the environment and to determine whether its lifetime is long enough for it to influence vegetation.

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The effect of climate on long-term changes in the crustacean zooplankton biomass of Lake Windermere, UK

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Fish predation is increasingly being viewed as one of the most important factors influencing the development of zooplankton populations in lakes^{1–3}. Marine zooplankton populations, on the other hand, are thought to be influenced primarily by the climate^{4,5}. We show here that year-to-year fluctuations in the biomass of crustacean zooplankton in Lake Windermere, in the United Kingdom, are strongly correlated with variations in water temperature, but poorly correlated with the abundance of the dominant planktivorous fish. This represents the first conclusive evidence of climatologically induced variability in a freshwater planktonic ecosystem. The links between climate and fisheries have recently been summarized by Cushing⁶, who suggests that climatic events regulate fish production through the matching or mismatching of their spawning with the period of available food. Our observations, reported here, suggest that year-to-year fluctuations in the biomass of crustacean zooplankton in Windermere are similarly influenced by the matching or mismatching of seasonal events in the zooplankton with the availability of algal food.

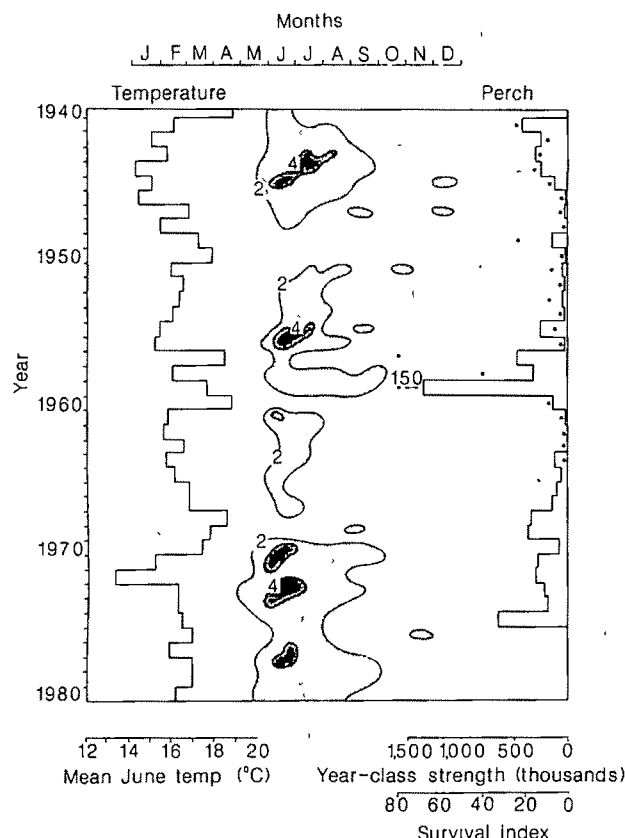


Fig. 1 The contour diagram shows the annual and seasonal variations in the biomass of crustacean zooplankton in the north basin of Windermere between 1940 and 1980. Biomass was estimated by a simple volumetric technique²³; the values shown are dry weights m^{-2} . The left-hand histogram shows the year-to-year variations in mean June temperature, and is based on weekly measurements at a central station in the north basin. All temperature measurements were taken in the top 5 m with a combined temperature and oxygen electrode (Mackereth). The right-hand histogram shows year-to-year variations in the year-class strength of perch between 1940 and 1976¹⁰ and includes estimates of a survival index for the years 1941–1964⁹. In 1976 the perch populations in Windermere were dramatically depleted by fungal disease¹⁵.

Windermere is the largest lake in the English Lake District (54°22'N, 2°56'W); it is divided into two distinct basins by an island and a region of shallows less than 5 m deep. The north basin is 8.05 km^2 in area and 64 m deep; the more productive south basin covers 16.78 km^2 and is 42 m deep. Long-term changes in the chemistry of Lake Windermere have been summarized by Sutcliffe *et al.*⁷, and Lund⁸ has published several accounts of the indigenous phytoplankton. The numbers and biomass of perch (*Perca fluviatilis* L.) in Windermere have been recorded and analysed every year since 1941^{9,10}, and the food preferences of the young perch have also been studied periodically^{11–13}. The crustacean zooplankton samples analysed here were collected from the north basin at fortnightly intervals between 1940 and 1980. All the samples were collected at the same mid-lake station by hauling a net vertically through the water column from a depth of 40 m. The conical net used had a mouth area of 700 cm^2 , a mesh size of 240 μm and efficiently captured all crustacea larger than first- or second-stage copepods. Occasional qualitative checks showed that the crustacean zooplankton populations in the north basin have changed little in the last 40 years. More detailed studies of samples collected in the last 10 years showed that, while the absolute abundance of the various species changed from year to year, there was no major shift in species dominance. The summer zooplankton crops are dominated by the cladoceran *Daphnia hyalina* var. *galeata* Sars and the cyclopoid copepods *Cyclops strenuus abyssorum* Sars and *Cyclops leuckarti* (Claus). Appreciable num-

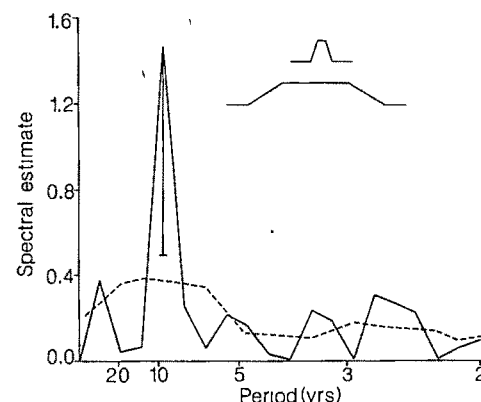


Fig. 2 High-resolution (—) and low-resolution (---) spectra for the 40-yr record of mean June temperature. The spectra were obtained by smoothing the raw spectra with the trapezium frequency window. The inset shows the bandwidths and smoothing window shapes for the two spectra. The main peak in the high-resolution spectrum is judged significant as its one-sided 95% confidence interval ($P < 0.05$) does not include the corresponding value in the low-resolution spectrum.

bers of the calanoid copepod *Diaptomus gracilis* Sars are present throughout the year, and two large cladoceran species *Leptodora kindti* (Focke) and *Bythotrephes longimanus* Leydig, appear for short periods in summer.

The contour diagram in Fig. 1 shows the seasonal and annual changes in crustacean zooplankton biomass that have occurred in the north basin between 1940 and 1980. It seems likely that the biomass of crustacean zooplankton has increased as a result of nutrient enrichment in recent years⁷, but the most obvious pattern is a quasi-cyclical variation with a period of about 10 years. Direct counts on a portion of this record show that the fluctuations in biomass principally reflect variations in the numbers of *D. hyalina*, the dominant herbivore. In Fig. 1 these fluctuations are compared with year-to-year fluctuations in mean June temperature and the recruitment and survival of perch. A. E. Irish (personal communication) has analysed the historical temperature records for Windermere¹⁴ and found that June is the only month in which a significant long-term autocorrelation is apparent. More detailed analyses of the Windermere temperature profiles show that these temperature variations are directly related to the timing of thermal stratification. The year-class strength records in Fig. 1 are a relative measure of the numbers of perch hatching each year and were obtained by trapping 2-yr-old fish. The assumptions inherent in these calculations have been discussed by Le Cren *et al.*⁹, who emphasize the relative nature of these estimates. A close examination of Fig. 1 suggests that the changes in crustacean zooplankton biomass are inversely related to mean June temperature, but bear very little relationship to either the year-class strength or the survival of perch. For example, the zooplankton fared poorly in 1950 and 1963 when there were few young perch in the lake, and reached quite high numbers in 1959 when perch were exceptionally abundant. The catastrophic incidence of perch disease in 1976¹⁵ also appears to have had little effect on crustacean zooplankton biomass. The very strong year-class of perch produced in 1959 may, however, have had some long-term effects, but the impact of older fish is difficult to assess as they do not feed extensively on zooplankton^{12,13}.

The overall interrelationships between crustacean zooplankton biomass, water temperature and perch recruitment are summarized by a series of multiple regressions in Table 1. Variations in the year-class strength of perch account for only 6.5% of the variation in crustacean zooplankton biomass, whereas variations in June water temperature account for almost 35% of the year-to-year variations in biomass. Such minor year-to-year variations in water temperature can have little direct effect on the survival and reproductive potential of the crustacean zooplankton; they are significant only insofar as they act as a surrogate for more

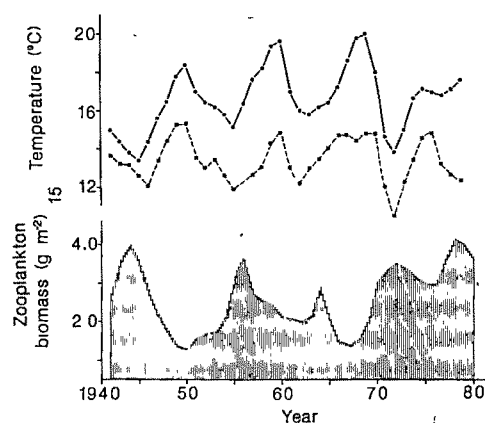


Fig. 3 Year-to-year variations in the mean summer (May to September) biomass of crustacean zooplankton in the north basin of Windermere, compared with year-to-year variations in mean June air (■) and water (●) temperature. The zooplankton biomass and water temperature data are taken from Fig. 1, and the air temperatures were obtained from a local meteorological stations. All the data were smoothed with a three-point-centred moving average with weights 0.25, 0.5 and 0.25.

fundamental weather-related factors such as wind-induced mixing.

Recently, several attempts have been made to resolve historically short-term (decades) periodicities in physical and biological data⁶. To assess the statistical significance of our temperature cycle, we have analysed our 40-year record using techniques of spectral analysis. Figure 2 shows the high-resolution and low-resolution spectra obtained from the 40-year record of mean June temperature. The peak at 9.8 yr in the high-resolution spectrum was tested by the bandpass filtering technique suggested by Hays *et al.*¹⁶ and judged significant at $P < 0.05$. The relationship between crustacean zooplankton biomass and this '10-year' temperature cycle is highlighted graphically in Fig. 3. This figure compares smoothed estimates of mean summer biomass with similar smoothed estimates of June air and water temperatures. There is a clear pattern of low zooplankton biomass in the summers following warm Junes, and high zooplankton biomass in the summers following cool Junes. The pattern is most pronounced for the temperature cycles recorded between 1940 and 1970. In the early 1970s, the weather pattern over northern Europe changed abruptly as northerly winds weakened and westerlies became dominant¹⁷. The less extreme biomass fluctuations recorded in Windermere over recent years may, in part, reflect this stabilizing influence. It is interesting that the water temperatures in Fig. 2 follow a more pronounced cycle than the air temperatures. This suggests that the onset of thermal stratification is strongly influenced by wind-induced mixing as well as by surface irradiance.

There seems little doubt that the quasi-cyclical events observed in Lake Windermere reflect a much wider pattern of climatic

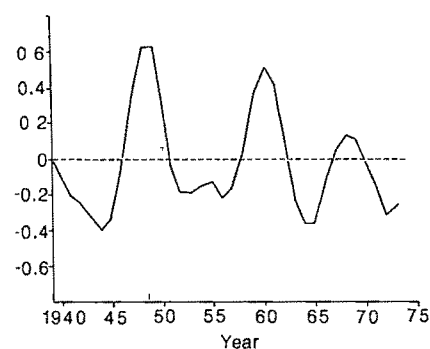


Fig. 4 The quasi-cyclical element of means of sea-surface temperature recorded in Marsden square 145D (45–50°N, 10–50°W) for the period 1940–1973²⁰. The plot was derived from the original data by the application, as a filter, of the second eigenvector of the matrix of serial correlations with a maximum lag of 7 years⁵.

variation. An approximately 10-yr periodicity in sea-surface temperature has been reported for several areas of the North Atlantic by Maximov *et al.*¹⁸ and for the English Channel by Southward *et al.*¹⁹. More recently, Colebrook and Taylor²⁰ have demonstrated a pronounced temperature cycle for Marsden square 145D (southern Celtic Sea and the northern Bay of Biscay). Figure 4 shows the annual mean anomaly of sea-surface temperature taken from their paper²⁰ for the period 1940–1973. The general similarities between the pattern of change in Windermere and the western sea approaches are fairly obvious. Colebrook and Taylor believe that this cyclical pattern is determined primarily by direct heat exchange but is again mediated by the meridional component of the surface winds.

Clearly, more information is needed to fully document the links between climate and zooplankton production in Windermere. Cushing⁶ has hypothesized that fish recruitment is strongly influenced by the matching or mismatching of the period of larval production with the period of available larval food. It seems likely that a comparable match/mismatch process controls the crustacean herbivore cycle in Windermere. Our preliminary observations certainly point to the importance of physical conditions in early summer, and suggest that these conditions influence the timing of biological events in the lake. Phytoplankton succession is known to be strongly influenced by weather-related mixing episodes²¹. Experimental studies in other lake systems²² also demonstrate that the availability of algal food influences the subsequent development of the crustacean plankton.

In Windermere, stratification is followed by the sedimentation of the spring diatom crop and the growth of a variety of nanoplankton. These small, rapidly growing species are an important source of food for filter-feeding crustacea who are less able to feed on the larger, slow-growing species that occur later in summer. The long-term nanoplankton records for Windermere

Table 1 Stepwise multiple regression of mean crustacean zooplankton biomass on mean June temperature (T) and on the year-class strength of perch (p)

	d.f.	Sum of squares	Mean square	Variance ratio
Regression on p alone	1	0.023	0.023	
Further inclusion of T in regression	1	0.532	0.532	17.16 ($P < 0.001$)
Regression on both p and T	2	0.554	0.277	
Regression on T alone	1	0.452	0.452	
Further inclusion of p in regression	1	0.102	0.102	3.29 (NS)
Regression on both p and T	2	0.554	0.277	
Residual	32	1.005	0.031	
Total	34	1.559	0.046	

d.f., Degrees of freedom; NS, not significant.

have not yet been analysed in detail (J.W.G. Lund, personal communication). Preliminary studies nevertheless suggest that the critical reproductive period for the crustacean zooplankton is more likely to coincide with the period of maximum food availability in 'cool' rather than 'warm' Junes. In warm Junes the preferred food species tend to appear earlier and may be in decline by the time the *Daphnia* start to reproduce rapidly.

The samples analysed in this paper have been collected, regularly, for a period of >40 yr. The authors would like to acknowledge their debt to all FBA staff who have been involved with this sampling programme. We thank A. H. Taylor and R. T. Clarke for critical comments on the manuscript, and M. A. Hurley and G. Tunncliffe-Wilson for statistical advice.

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Taxonomic affinities of the immature hominid crania from Hadar and Taung

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The description¹ of the AL 333-105 cranium from the East African Pliocene provides a new opportunity to re-examine the suggestion²⁻⁴ that the Taung child may be a robust rather than a gracile form of early hominid. Comparisons of these two crania with the skulls of living and fossil hominoids indicate that AL 333-105 possesses autapomorphic features associated with *Paranthropus* masticatory hypertrophy, and provide additional evidence for the existence of this lineage in the Hadar Formation. Here, I report a study of the facial morphology of the Taung specimen which, together with recent observations on its dentition⁵, provides strong evidence against the allocation of the Taung child to the *Paranthropus* clade. The identity of the specimen is recognized as being in the *Homo* lineage.

The comparison of 71 crania of *Gorilla*, 85 of *Pan troglodytes*, 67 of *Pan paniscus*, 40 of *Pongo* and 164 of *Homo sapiens* demonstrates that nasal bone outline and the relationships of the frontal, nasal and maxillary bones are highly consistent within and between these taxa. Typically, in great apes of both sexes and all age groups, nasion and glabella are closely approximated on the supraorbital torus (Fig. 1). The nasal bones are widest immediately superior to the piriform aperture and they converge superiorly. The relatively large maxillary processes of the frontal bones project inferiorly beside the nasal bones,

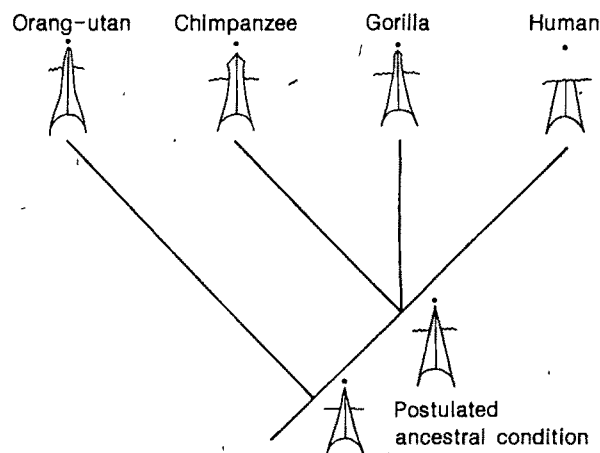


Fig. 1 Cladogram of extant hominoid taxa, illustrating the nasal bones and superior margin of piriform aperture in *norma frontalis*. Figures (to scale) indicate the differences between the nasal outlines and the relative positions of nasion to glabella (•).

placing the frontomaxillary sutures in a roughly transverse plane below the level of nasion.

The significance and primitive nature of this pattern in the great apes are further indicated by: (1) its presence in the orang-utan where the inter-orbital region is derived; (2) its existence in the Miocene ape *Sivapithecus*⁶; and (3) the minimal variation found between infant and adult specimens. The third observation is particularly strong evidence that great-ape nasal morphology is symplesiomorphic.

In contrast, the morphology of the nasal region in *H. sapiens* is derived in the reduction or absence of the maxillary processes of the frontal bone as seen in a frontal view, and in the location of nasion relative to glabella (Fig. 1). In *H. sapiens*, the frontonasal and frontomaxillary sutures are linear and continuous below an anteriorly projecting supraorbital torus, and nasion is removed from its primitive association with the supraorbital torus and glabella (Fig. 1).

The ontogenetic evidence for a consistent association of these features in *H. sapiens* is compelling. Fetal human crania exhibit the adult condition even in the absence of a supraorbital torus⁷. The data collected in this study from an ontogenetic series of *H. sapiens* crania⁸ agree with the developmental data from other hominoid taxa. Thus, nasal morphology is useful in determining the phylogenetic affinities of both adult and immature fossil crania.

The taxonomic relevance of nasal morphology to early hominid systematics is further increased by the existence in *Paranthropus* of a third derived pattern (Fig. 2a-d). In *Paranthropus*, the lateral margins of the nasal bones are derived from the primitive hominoid pattern in the way they diverge superiorly. However, the primitive relationships between: (1) nasion and glabella; and (2) the frontonasal and frontomaxillary sutures are retained. Nasion and glabella are closely approximated well above the points where the frontomaxillary sutures meet the nasal bones, and the nasomaxillary sutures are either parallel or divergent superiorly. The frontal profile of *Paranthropus* nasal bones is described here as a 'keystone' pattern.

Loading of the anterior dentition during occlusion has been shown⁹ to generate intense forces in the face that are transmitted through the frontal processes of the maxillae to the supraorbital torus, where they are dissipated in the cranial vault. In hominoids with a primitive triangular nasal profile (Fig. 1), the frontal processes of the maxillae act as simple pillars that transmit force directly to the beam-like supraorbital torus. The derived keystone condition in *Paranthropus* is an adaptation that more evenly distributes anterior occlusal forces through the supraorbital torus to the cranial vault.

A supported arch with its central keystone functions to distribute overlying weight evenly to laterally located pillars. The

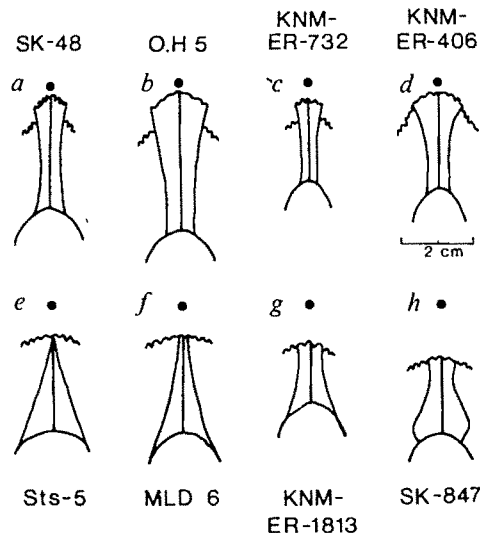


Fig. 2 Nasal bones and superior margin of piriform aperture in specimens of *Paranthropus* from Swartkrans (a), Olduvai Gorge (b) and Koobi Fora (c, d); *Homo* (*Australopithecus*) from Sterkfontein (e), Makapansgat (f) and Koobi Fora (g); and *Homo* (*Homo*) from Swartkrans (h). *Norma frontalis* reconstructions indicate the differences in nasal profile, and the relative position of glabella (•) to nasion and to the plane between the points where the two frontomaxillary sutures meet the nasal bones.

nasal region of *Paranthropus* functions in a similar manner except that the compressive forces distributed in its arch-like structure are applied by the pillars rather than by overlying weight. In the vertical face of *Paranthropus*, compressive forces generated by anterior dental loading are transmitted through the frontal processes of the maxillae (the pillars) to the nasal keystone (the arch) where they are evenly distributed to the supraorbital torus. The adaptive significance of this arrangement is probably associated with paranthropine masticatory evolution. In *Paranthropus*, the premolars became increasingly involved with heavy chewing and were enlarged anteriorly at the expense of the canines and incisors. As a result, the forces generated during anterior dental loading would have been significantly increased¹⁰.

Hominid supraorbital morphology has been studied¹¹ with respect to the functional relationship between facial prognathism and stress resistance by the torus. Russell¹¹ concluded that a flat face optimizes the transmission of vertical bite force from the anterior dentition to the supraorbital region. Similarly, the flat *Paranthropus* face has been described^{9,12,13} as an adaptation to the anterior enlargement of the premolars and their increased role in mastication.

Although the frontal profile of the nasal bones in *Paranthropus* is derived relative to the postulated common hominid ancestor, the gracile early hominids (Fig. 2e-h) retain the primitive hominoid profile but are derived in the location of nasion relative to glabella. In Sts. 5, MLD. 6 and other members of the *Homo* lineage, the nasal bones appear truncated below the supraorbital torus, with the frontomaxillary and frontonasal sutures oriented close to a horizontal plane below the variably inflated glabellar region. The extent of this superior nasal truncation in Sts. 5 and MLD. 6 is somewhat less derived than the condition observed in later *Homo* forms. However, in both specimens, nasion is at the base of the supraorbital torus well inferior to glabella.

The nasal region has been described¹ for AL 333-105. This specimen was collected at the same Hadar Formation locality as AL 333-45, which has been shown¹⁴⁻¹⁷ to have paranthropine basicranial characteristics. The data used to reconstruct the AL 333-105 specimen (Fig. 3a) are from Kimbel *et al.*¹. However, whereas these authors conclude that the size and form of the nasal bones are 'unusual', the present results indicate the presence of a paranthropine type of pattern. Comparison of the AL 333-105 nasal morphology with the postulated primitive hominid

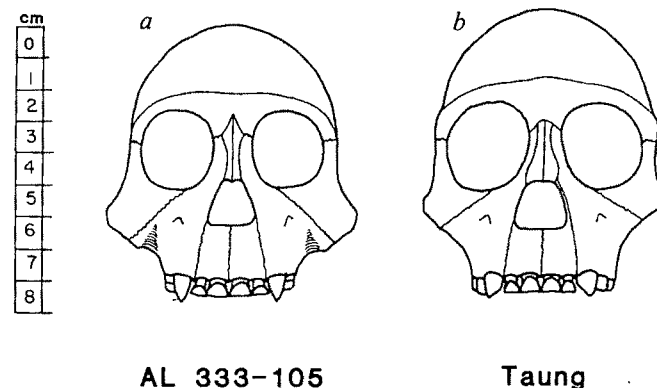


Fig. 3 *Norma frontalis* reconstructions of juvenile crania AL 333-105 from the Hadar Formation (a) and from Taung (b).

condition (Fig. 1) indicates a highly derived rather than a primitive morphology¹⁸.

Fortunately, the immature age of AL 333-105 does not diminish the taxonomic relevance of its nasal morphology. Continued facial growth could only further reduce the combined nasal breadth at the inferior half of the nasal bones where they are overlapped laterally by the frontal processes of the maxillary bones¹⁹. Thus, it is reasonable to conclude that the nasal profile of an adult AL 333-105 would bear an even closer resemblance to known adult specimens of *Paranthropus* (Fig. 2a-d) than does the actual condition observed in the juvenile skull.

The paranthropine nasal pattern in AL 333-105 is particularly pertinent to the suggestion²⁻⁴ that the Taung skull (Fig. 3b) may be a form of *Paranthropus*, based on its apparently young geological age. However, the affinity of the Taung specimen must be based solely on its morphological characteristics²⁰ and not on an undefined method of chronotaxonomy. The phylogenetic comparisons of the Taung child conducted in this study identifies the derived nasal pattern of the *Homo* lineage and does not support the inclusion of the Taung specimen with AL 333-105 in the *Paranthropus* clade.

Another comparative study of the Taung child⁵ has concluded that it does not possess the derived dental features that characterize *Paranthropus*. Grine's⁵ conclusions assume added significance in view of suggestions²⁻⁴ that Taung represents the terminal phase of paranthropine evolution. It could be argued that an immature individual of the earliest paranthropines might not exhibit the full suite of cranial or dental specializations seen in later forms. However, it is unlikely that the terminal individuals of this clade would exhibit few, if any, of the specializations seen in its ancestors.

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Phorbol ester and sperm activate mouse oocytes by inducing sustained oscillations in cell Ca^{2+}

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The parthenogenetic activation of mouse oocytes by several agents is accompanied by a large rise in the intracellular calcium concentration ($[\text{Ca}^{2+}]_i$)¹. The tumour-promoting phorbol ester 12-*O*-tetradecanoyl phorbol acetate (TPA) activates some cellular processes which are also activated by raised $[\text{Ca}^{2+}]_i$ (ref. 2). We therefore tested TPA on mouse oocytes and found it to be a potent parthenogenetic activating agent. Recent reports suggest that TPA mimics endogenous diacylglycerol³ and can stimulate cells by activating protein kinase C without raising $[\text{Ca}^{2+}]_i$ (refs 4, 5). We have now measured $[\text{Ca}^{2+}]_i$ with aequorin in mouse oocytes treated with TPA. Sustained oscillations in $[\text{Ca}^{2+}]_i$ appeared in those oocytes which were subsequently activated. We also measured $[\text{Ca}^{2+}]_i$ during fertilization. The response began with $[\text{Ca}^{2+}]_i$ transients as reported previously¹, but the transients continued in a regular pattern for 4 h. There was no rise in $[\text{Ca}^{2+}]_i$ of the type induced by activating agents such as ethanol. One component of the fertilization response appears to be related to the TPA-induced oscillations.

Unfertilized superovulated oocytes incubated with TPA, or the active analogue 4 β -phorbol 12,13-dibutyrate (PDB), were found to be activated when examined after 5 h, but the relatively inactive 4-*O*-methyl derivative of TPA was ineffective at the

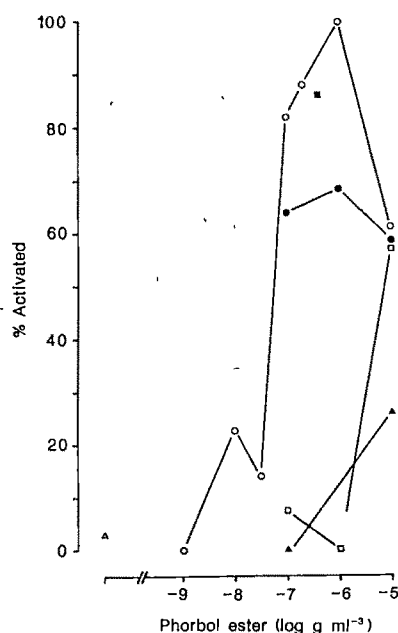


Fig. 1 Activation of mouse oocytes by phorbol esters. Superovulated mouse oocytes (MF1; Olac, Bicester) obtained 16–18 h after the injection of human chorionic gonadotropin (HCG), were incubated in Whittingham's bicarbonate-buffered medium 16 plus the test agent, fixed after 5 h and scored under Nomarski optics for the presence of pronuclei. ○, TPA, 5 h; ●, TPA, 10 min; □, 4-*O*-methyl derivative of TPA, 5 h; ■, phorbol 12,13-dibutyrate, 5 h; ▲, dimethyl sulfoxide (DMSO) control, 5 h; Δ, medium-only control. The data are combined results from six experiments with a total of 665 oocytes. The phorbol esters (Sigma) were dissolved in DMSO (1 mg ml⁻¹). (CBA × C57BL/6)F₁ oocytes treated with 100 ng ml⁻¹ TPA for 5 h developed to morulae and blastocysts (54%, 93/171) in similar proportion to diploid controls treated¹¹ with 7% ethanol for 7 min and cytochalasin D for 5 h (53%, 67/126).

same concentrations⁶ (Fig. 1). In most (94%) of the activated oocytes the second meiotic division was suppressed. This is unusual for artificial activating agents, which usually induce a mixture of activation types⁷, and can be contrasted with fertilization, which almost invariably produces a second polar body.

Free $[\text{Ca}^{2+}]_i$ in the oocytes was measured with aequorin during treatment with a maximally activating concentration of TPA (200 ng ml⁻¹). After a variable delay of 30–80 min, small oscillations in $[\text{Ca}^{2+}]_i$ appeared in some oocytes (6/12 oocytes, observed for at least 2 h). A higher concentration of TPA (1 μg ml⁻¹) induced oscillations within 10 min (3/4 oocytes). The oscillations continued for up to 3 h (Fig. 2). All oocytes with Ca^{2+} oscillations which were examined later (five oocytes)

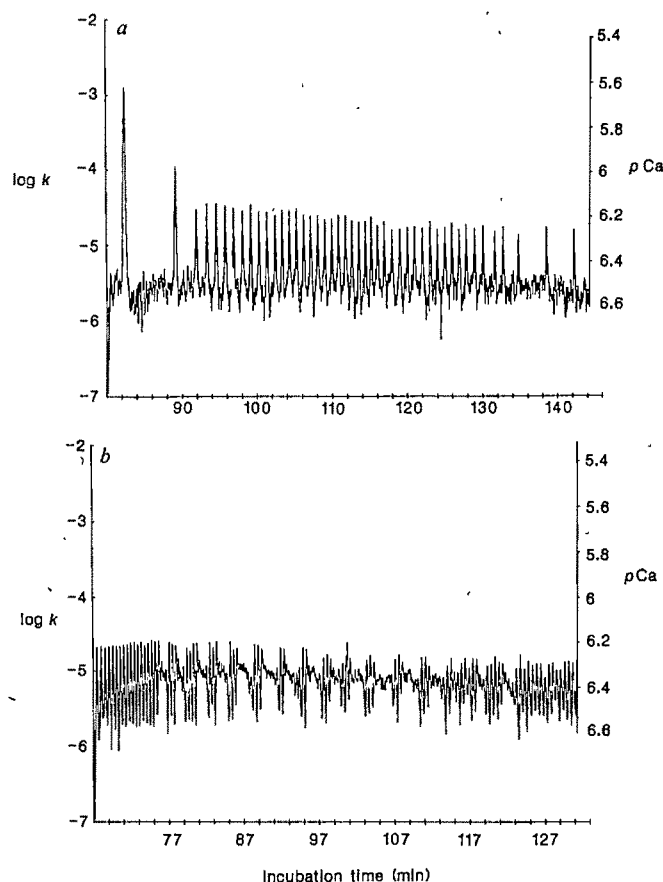


Fig. 2 The $[\text{Ca}^{2+}]_i$ response to 200 ng ml⁻¹ TPA in single mouse oocytes. *a*, Onset of $[\text{Ca}^{2+}]_i$ oscillations; *b*, section of oscillations showing the appearance of a 'bursting pattern' in the oscillations. Oscillations had begun in this oocyte 12 min earlier than shown, and continued for a total of 200 min. A similar bursting pattern of oscillations was seen in one other oocyte. Log *k* is log₁₀ of the fraction of aequorin consumed per s.

Methods. Mouse oocytes, taken 15–18 h after the HCG injection from (C57BL/6 × CBA)F₁ females, were injected with the photo-protein aequorin and placed in glass microslides in a chamber at 37°C under a photomultiplier as described previously^{11,12}. The oocytes were treated with 0.5 μg ml⁻¹ cytochalasin D (Sigma) for ~1 h before injection. Photon pulses were passed through a Nuclear Enterprises photon counting system or an EMI amplifier-discriminator (type C601). Photon counts were sampled every 50 ms and stored in a Sirius 1 microcomputer. Background was subtracted from the counts, which were then normalized, smoothed exponentially (time constant 5 s) and calibrated¹² to give the plots shown here. The oocytes were examined at the end of the experiments with Nomarski optics (Nikon). Both oocytes from the illustrated experiments were activated. $[\text{Ca}^{2+}]_i$ was measured until pronuclei appeared. No change in $[\text{Ca}^{2+}]_i$ other than the oscillations could have induced the activation. The medium was Whittingham's bicarbonate-buffered medium 16, modified by replacing lactate with HEPES pH 7.4, and gassed with 5% CO₂ in air. For the fertilization experiments this medium was further modified to raise pH to 7.7 and the bovine serum albumin content fivefold to 20 mg ml⁻¹.

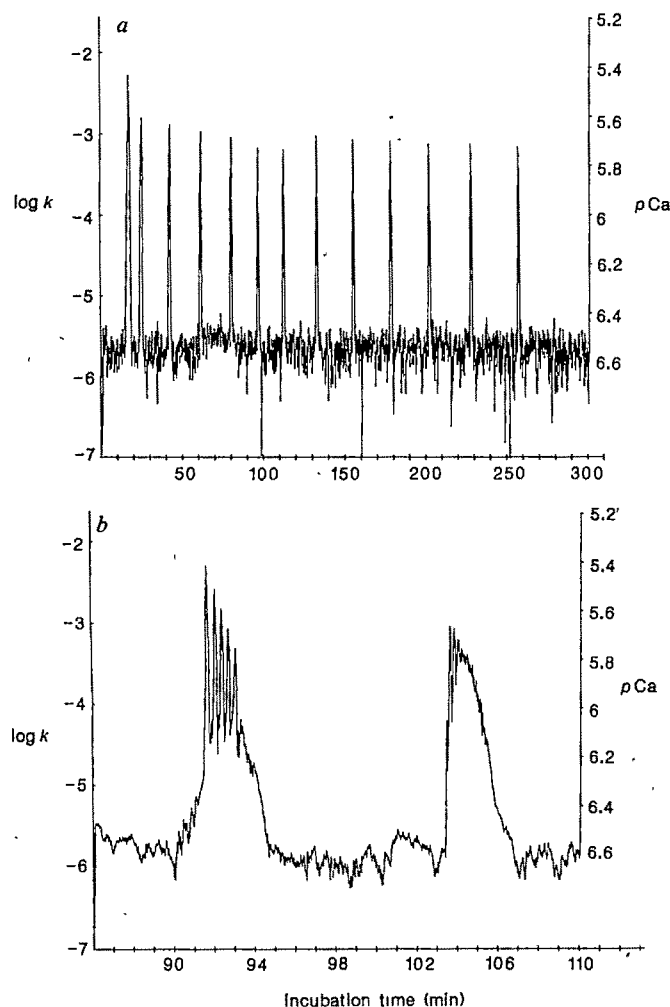


Fig. 3 The $[Ca^{2+}]_i$ response during fertilization in single mouse oocytes. *a*, A typical series of 13 $[Ca^{2+}]_i$ transients showing their sustained periodicity. *b*, The first two transients in another oocyte, showing fast oscillations on each transient (time constant 30 s for resting level, reduced to 0.5 s for the transients). The oocyte zona pellucida was removed by digestion with α -chymotrypsin (0.03%) after the aequorin injection. Preincubated sperm from (C57BL/6 $\times$ CBA)E₁ males were added to the microslides after an initial measurement period.

were activated, with one or two female pronuclei and no second polar body; oocytes which did not develop Ca^{2+} oscillations remained unactivated (three oocytes).

The Ca^{2+} oscillations had periods of between 17 and 35 s. In each sustained series of oscillations, $[Ca^{2+}]_i$ reached $5\text{--}7 \times 10^{-7}$ M, assuming a uniform distribution of $[Ca^{2+}]_i$. However, if $[Ca^{2+}]_i$ rose in only part of the cell volume (10–30%), then locally it could have exceeded $1 \mu\text{M}$. $[Ca^{2+}]_i$ often seemed to oscillate below and above an underlying $[Ca^{2+}]_i$ concentration which could either remain constant (Fig. 2*b*), or rise or fall (data not shown). Raising $[Ca^{2+}]_i$ in TPA-treated oocytes with ethanol (7%) or benzyl alcohol (0.4%) could induce oscillations, if none were yet present, and these oscillations were superimposed on a large $[Ca^{2+}]_i$ rise of the type previously reported¹. Slower $[Ca^{2+}]_i$ rises, produced by raising external Ca^{2+} (fivefold) or occurring spontaneously, extinguished the oscillations within a few minutes. During corresponding slow falls in $[Ca^{2+}]_i$, oscillations sometimes reappeared. This suggests that calcium itself can both stimulate and inhibit the oscillations over the course of several cycles. Such a dual influence may explain the regular appearance and disappearance of the oscillations seen in Fig. 2*b*.

We measured $[Ca^{2+}]_i$ in four zona-free oocytes during fertilization. The $[Ca^{2+}]_i$ response was complex but remarkably similar in all four cells, consisting of a regular series of large Ca^{2+}

transients (reaching 2×10^{-6} M) which continued for 4 h (Fig. 3). The interval between the first two transients was about 10 min and that between the following transients was about 20 min. There were 13 transients in three oocytes and 15 in the fourth. Superimposed on the transients were faster oscillations, with a period of about 20 s on the first transients and about 10 s on the rest. The oscillations on the first transients reached 4×10^{-6} M. After the last transient there was no further change in $[Ca^{2+}]_i$ (measured for at least a further 10 h). All four oocytes were subsequently found to be fertilized, with two pronuclei and a second polar body.

The oscillations on the fertilization transients have several features in common with the TPA-induced oscillations. For example, the frequencies are similar and the oscillations appear to modulate $[Ca^{2+}]_i$ both up and down from what it would otherwise have been. Small oscillations in membrane potential (with period ~ 20 s) have been recorded during fertilization in mouse oocytes⁸; larger recurrent hyperpolarizations occur in hamster oocytes⁹ and repetitive diphasic membrane potentials have been recorded in rabbit oocytes¹⁰. These may be a consequence of $[Ca^{2+}]_i$ oscillations like those we have observed on the first fertilization transient.

The TPA- and sperm-induced $[Ca^{2+}]_i$ responses reported here are significantly different from each other, and from the alcohol-induced $[Ca^{2+}]_i$ response¹. (We have confirmed in this experimental series that ethanol and benzyl alcohol induce large stepwise exponential rises in $[Ca^{2+}]_i$ in oocytes which subsequently became activated (four oocytes).) These three types of $[Ca^{2+}]_i$ response are all capable of activating the egg. The production of a second polar body, however, seems to be more critically dependent on time-course and amplitude of the $[Ca^{2+}]_i$ response. A similar variety of receptor-mediated $[Ca^{2+}]_i$ responses may occur in other cells in which $[Ca^{2+}]_i$ controls several cellular processes, providing extensive possibilities for selective operation of the $[Ca^{2+}]_i$ messenger system.

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Immunocytochemical localization in rat brain of a prolactin release-inhibiting sequence of gonadotropin-releasing hormone prohormone

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The structure of a precursor protein for gonadotropin-releasing hormone (GnRH) of relative molecular mass 10,000 has recently been deduced from cloned complementary DNA sequences derived from human placental messenger RNA¹. The 56-amino-acid peptide representing residues 14–69 of this prohormone exhibits potent inhibition of prolactin secretion². To investigate whether the same prohormone is synthesized in mammalian brain and describe the anatomical distribution of the prolactin-inhibiting region of this

Fig. 1 *a*, Structure of human proGnRH (1-69) in single-letter amino-acid code. Underlined sections represent the decapeptide GnRH and proGnRH-40-53 synthesized for antibody production. *b*, Radioimmunoassay with antiserum 39A showing displacement of iodinated proGnRH-40-53 by increasing concentrations of proGnRH-40-53 standard or rat hypothalamic extract.

Methods. Sera from three rabbits immunized with proGnRH-40-53 conjugated to bovine serum albumin using *N*-(maleimidobutyryloxy)-succinimide were screened by radioimmunoassay. Serum from one rabbit (39A) contained antibodies to proGnRH-40-53 and recognized proGnRH-14-69 but not GnRH or proGnRH-14-24. The assay was carried out as described for adrenocorticotrophic hormone¹⁹ with some modifications. The *p*-hydroxyphenylmaleimide derivative of proGnRH-40-53 was radioiodinated using chloramine T. Antiserum 39A was used in a 1:10,000 final dilution. Bound and free radioactivity were separated by centrifugation after addition of charcoal to the assay incubation. Hypothalami from 10 female Sprague-Dawley rats (200-250g) were homogenized in 5 volumes of phosphate buffered saline solution pH 7.4. After centrifugation (15 min, 800g) 50 μ l aliquots of the supernatant were assayed by radioimmunoassay at the indicated dilutions (undiluted, 1:10, 1:100).

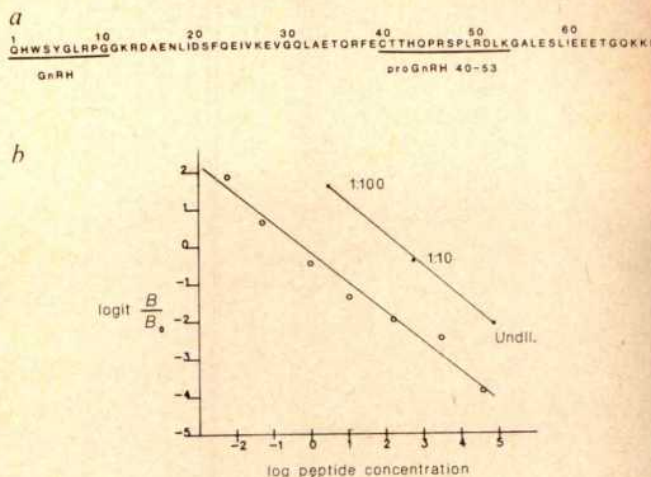
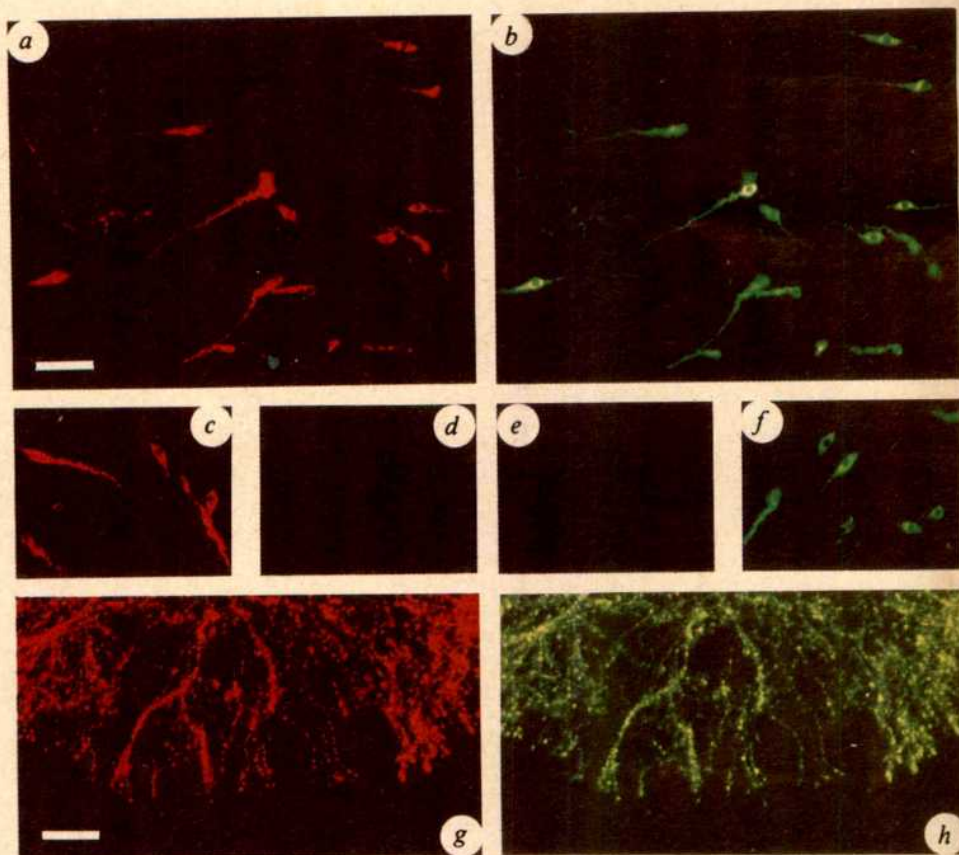


Fig. 2 Dual immunofluorescence staining with anti-proGnRH-40-53 and GnRH antisera. *a, b*, A coronal section through the diagonal band of Broca demonstrating perikarya stained for both proGnRH-40-53 (*a*) and GnRH (*b*). *c, d*, Adsorption control with GnRH (10 μ g ml⁻¹). ProGnRH-40-53 antibody reacts strongly with perikarya in the diagonal band (*c*), whereas no staining is visible in the same region with GnRH antibody (*d*). *e, f*, Adsorption control with proGnRH-40-53 (10 μ g ml⁻¹). GnRH-immunoreactive cells are abundant (*f*) while no reaction is detected with proGnRH antibody (*e*). *g, h*, Coronal section through median eminence demonstrating colocalization of proGnRH-40-53 (*g*) and GnRH (*h*) within axonal terminals. Scale bar, 50 μ m for *a-f*, 40 μ m for *g* and *h*.

Methods. Sprague-Dawley rats were fixed by intracardiac perfusion with 4% paraformaldehyde at pH 7.2. Cryostat or vibratome sections of fixed tissue were stained sequentially for the localization of proGnRH-40-53 and GnRH according to the following protocol. Antiserum to proGnRH-40-53 prepared as described in Fig. 1, was preadsorbed with rat liver powder and used at a dilution of 1:2,000. Antiserum DB-1 against a Orn⁸-GnRH conjugated to bovine serum albumin was generated in rabbits, affinity purified on D-Lys⁶-GnRH-agarose, biotinylated and used at 1-2 μ g ml⁻¹. All incubation solutions contained 0.1M Tris, pH 7.2, 0.9% NaCl, 1.0% bovine serum albumin, 0.1% Triton and 0.1% Merthiolate. Tissue sections were first incubated with anti-proGnRH-40-53 for 6 h at 20 °C or 24 h at 4 °C followed by rhodamine labelled goat anti-rabbit IgG (10 μ g ml⁻¹) for 4 h at 20 °C. To saturate free binding sites on the anti-rabbit IgG, sections were incubated in 20% normal rabbit serum for 1 h. All subsequent incubations were at room temperature and contained 20% normal rabbit serum. Biotinylated anti-GnRH was applied as the second primary antibody (12 h) and was recognized by fluorescein-avidin DCS (Vector, 2.5 μ g ml⁻¹, 1 h). The fluorescent signal associated with the anti-GnRH antibody was then amplified by incubation with biotinylated goat anti-avidin (Vector, 5 μ g ml⁻¹, 1 h), followed by a second layer of fluorescein-avidin (5 μ g ml⁻¹, 1 h). Sections were mounted in glycerol and examined with a Zeiss epifluorescence microscope with fluorescein and rhodamine filter sets.



molecule, we have generated antiserum to a synthetic peptide containing residues 40-53 of the human placental precursor. We report here that a substance recognized by this antibody is present in GnRH-containing neurones of the rat brain and appears to coexist with GnRH in secretory granules of nerve terminals in the median eminence. These results indicate homology between hypothalamic and placental prohormones for GnRH and are consistent with the suggestion elsewhere in this issue² that a prolactin-inhibiting factor (PIF) is generated from this prohormone and cosecreted with GnRH by nerve terminals in the median eminence.

Antiserum was generated in rabbits to a synthetic peptide comprising residues 40-53 of the human placental precursor to GnRH (Fig. 1a). This peptide (referred to as proGnRH-40-53) represents the mid-portion of the 56-amino-acid peptide which exhibits potent inhibition of prolactin secretion². Radioimmunoassay using this serum revealed the presence in rat brain of an antigen characterized by parallel displacement with synthetic proGnRH-40-53 (Fig. 1b).

A double-label immunofluorescence technique demonstrated the coexistence of GnRH and proGnRH-40-53 in neurones of

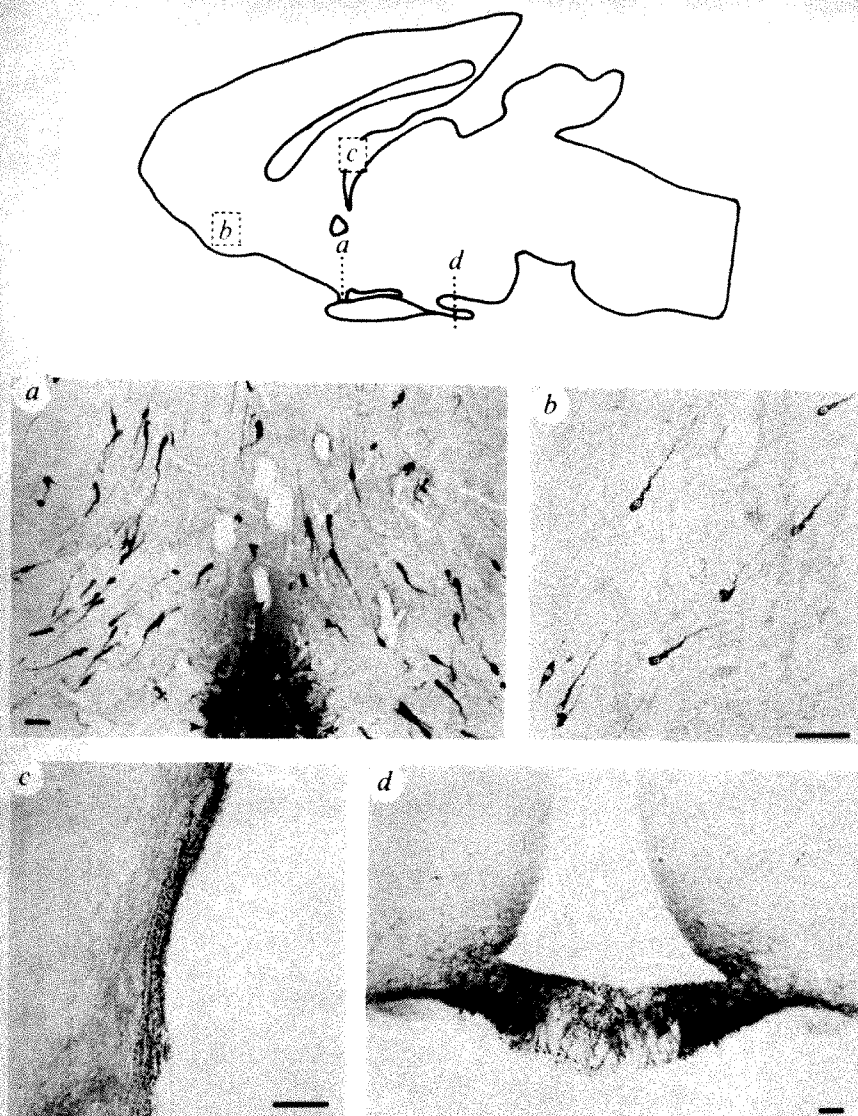


Fig. 3 Distribution of proGnRH-40-53 immunoreactivity in rat brain. Schematic diagram of sagittal section through rat brain depicts the location of micrographs *a-d*. *a*, Coronal section demonstrating numerous immunoreactive perikarya in the diagonal band of Broca and a dense terminal field in the OVLT (arrowheads). *b*, Sagittal section displaying immunoreactive perikarya in the parolfactory region. *c*, Sagittal section depicting immunoreactive fibres along the surface of the third ventricle in the region of the subfornical organ. *d*, Coronal section through median eminence with numerous terminals immunostained for proGnRH-40-53. Scale bars, 50 μ m.

Methods. Brains from male and female Sprague-Dawley rats were fixed for immunocytochemistry by intracardiac perfusion of the animals with 4% paraformaldehyde. GnRH and proGnRH-40-53 immunoreactivities were detected in alternate vibratome sections by a modification of the avidin-biotin peroxidase complex (ABC) method²⁰. Antibody to proGnRH-40-53 (39A) was prepared as described above. Antisera to GnRH were provided by B. Kerdelhue (4,150-11) and S. Vigh (16). Primary antibody incubations (4,150-11, 1:4,000-1:8,000; 16, 1:5,000-1:10,000; 39A, 1:1,000-1:2,000) were conducted for 12-24 h at 4°C. Antibodies and ABC reagents (Vector) were diluted in Tris buffered saline at pH 7.2 containing 1% normal goat serum, 0.1% Triton X-100 and 0.1% gelatin. In some instances, primary antibodies (diluted 1:10 in 0.1 M Tris) were preadsorbed with rat liver powder (Cappel Labs) or rate cerebellum powder for up to 24 h at 4°C to reduce non-specific staining. Controls for specificity of staining with antibody to proGnRH-40-53 were conducted by preincubation of each ml of diluted serum with 10 μ g synthetic proGnRH-40-53. The only nonspecific interaction of proGnRH-40-53 antiserum noted was with a population of multipolar cells in the olfactory tubercle.

the septal-preoptic area (Fig. 2*a, b*). Perfect correspondence was observed between cells immunoreactive for the two antigens. Specificity of staining was demonstrated by preadsorption of the primary antibodies with synthetic GnRH or proGnRH-40-53 (Fig. 2*c-f*).

Previous studies on the biosynthesis of GnRH have revealed high relative molecular mass immunoreactive forms of the hormone in mammalian brain³⁻⁵. Our findings indicate that GnRH-containing neurones of rat brain produce a peptide homologous in sequence to the M_r 10,000 (10K) human placental precursor for GnRH. This apparent homology complements studies suggesting the presence of a single gene for GnRH^{1,6}. Thus, GnRH synthesis in human brain is likely to occur via a precursor identical to the 10K sequence present in human placenta.

To characterize the anatomical distribution of the prolactin release inhibiting sequence of GnRH prohormone, alternate sections through the entire rat brain were stained with antibodies to GnRH or proGnRH-40-53 by immunoperoxidase labelling (Fig. 3). Similar to previous reports for GnRH-immunoreactive perikarya in the rat⁷⁻¹⁰, GnRH and proGnRH-40-53-like antigens were localized to smooth-contoured bipolar neurones and irregularly outlined monopolar cells residing in the septo-preoptic region. The distribution of cells immunoreactive for proGnRH-40-53 was identical to that observed for GnRH and included the medial septal nucleus, the diagonal band of Broca (Fig. 3*a*), the nucleus triangularis septum, the medial preoptic area, the anterior hypothalamus and the parolfactory region near the midline which extends from the anterior commissure to the olfactory bulb (Fig. 3*b*). The largest concentration of

immunoreactive perikarya was located in the diagonal band of Broca near the organum vasculosum of the lamina terminalis (OVLT).

The pattern of immunoreactivity observed in rat brain with antiserum to proGnRH-40-53 was not restricted to neuronal perikarya, but also appeared in axons and terminals. Many axons stained for proGnRH-40-53 were visible throughout septal, preoptic and hypothalamic regions. Bundles of immunoreactive axons were observed along the ependymal lining of the third and lateral ventricles (Fig. 3*c*). Dense networks of terminals appeared in the OVLT (Fig. 3*a*) and median eminence (Fig. 3*d*). Extrahypothalamic projections to the olfactory bulb, amygdala, habenula and mesencephalic central grey were noted. In every region examined, the appearance of projections immunoreactive for proGnRH-40-53 was identical to that observed for GnRH. Double-label immunofluorescence experiments on tissue from septo-preoptic and hypothalamic regions (Fig. 2*g, h*) revealed perfect correspondence between axons immunoreactive for GnRH and proGnRH-40-53. The presence of proGnRH-40-53-like antigen in axons and nerve terminals as well as perikarya is consistent with earlier immunocytological evidence that cleavage of GnRH from its prohormone occurs in axonal varicosities and terminals¹¹ as described for other neuropeptide systems¹²⁻¹⁴.

At the ultrastructural level, large dense-cored vesicles immunoreactive for proGnRH-40-53 were observed in terminal and preterminal varicosities of the median eminence (Fig. 4). Within terminals immunoreactive for proGnRH-40-53, virtually every vesicle exhibited deposition of reaction product. These

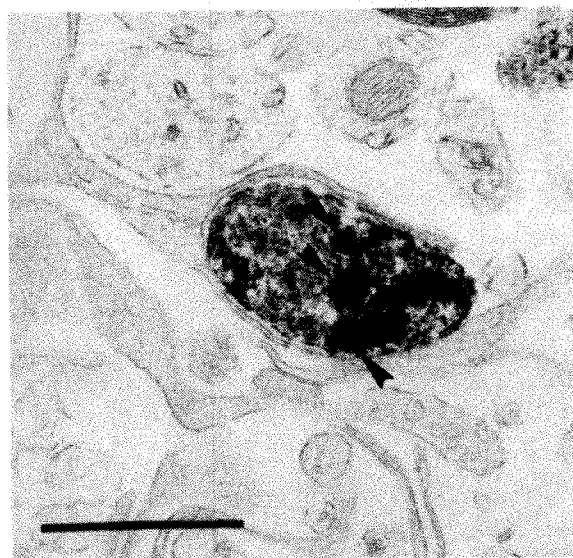


Fig. 4 A proGnRH-40-53 immunopositive axonal varicosity in the median eminence. Reaction product is associated with large vesicles (arrows). Scale bar, 1 μ m.

Methods. Tissue fixation and immunocytochemical procedure was identical to that described for light microscopy with the following exceptions: 0.5% glutaraldehyde was included in the fixative and triton concentrations of 0.025% rather than 0.1% were used throughout the immunocytochemical procedure. After development of reaction product, the tissues were prepared and examined by electron microscopy as described previously²¹.

results are similar to earlier observations on GnRH-immunoreactive vesicles in nerve terminals of rat median eminence¹⁵⁻¹⁷. Given the complete overlap between terminals immunoreactive for GnRH and proGnRH-40-53 in the median eminence (Fig. 2g, h), coexistence of these peptide sequences within individual vesicles is indicated.

Extracts of hypothalamic tissue have long been known to contain prolactin release inhibiting activity¹⁸. As the 56-amino-acid carboxy-terminal GnRH prohormone fragment exhibits potent inhibition of prolactin release², our data are consistent with the idea that a prolactin release-inhibiting peptide is processed from the GnRH prohormone and secreted from terminals in the median eminence into the pituitary portal circulation. The coexistence of this peptide with GnRH in extra-hypothalamic axonal projections suggests additional roles for this substance in the regulation of neuronal activity and/or behavioural processes.

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Antigen-dependent increase in cytosolic free calcium in specific human T-lymphocyte clones

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Calcium has been implicated as an intracellular messenger in the cellular response to various external stimuli. Exposure of lymphocytes to various mitogens and lectins results in rapid transmembrane calcium fluxes¹⁻³ and increased cytoplasmic calcium concentrations ($[Ca^{2+}]_i$)⁴⁻⁷. It is not clear, however, whether the mechanisms by which these non-physiological stimuli activate cells are related to those involved in antigen-specific activation. We have now used antigen-specific T-cell clones^{8,9} to study changes in $[Ca^{2+}]_i$ associated with specific activation and show here that these cells respond specifically in the presence of antigen and antigen-presenting cells (APC) with increased $[Ca^{2+}]_i$ and that this increased $[Ca^{2+}]_i$ shows the same genetic restrictions as are seen in the proliferation assay^{8,10,11}. The kinetics of the $[Ca^{2+}]_i$ response to antigen indicate that antigen undergoes a time-dependent processing step as a prerequisite for recognition by T cells, as has been shown for T-cell proliferative responses¹²⁻¹⁴, but that the $[Ca^{2+}]_i$ response to processed antigen is extremely rapid. The close correlation between changes in $[Ca^{2+}]_i$ and cell activation resulting in proliferation suggests that Ca^{2+} may act as an intracellular messenger in antigen-specific responses.

Until recently, it has been impossible to study $[Ca^{2+}]_i$ directly in cells responding to antigen. The fraction of cells that respond specifically to antigen in an unselected population is small compared with that activated by mitogens, and even substantial changes in $[Ca^{2+}]_i$ in this subpopulation would not be detectable using current techniques which measure the average $[Ca^{2+}]_i$ in a population. To overcome this problem we have derived tetanus toxoid (TT)-specific human T-cell clones from an immunized HLA-DR2, 7 donor by modifications of previously described techniques^{8,10,15}. Peripheral blood mononuclear cells (PBM) were restimulated *in vitro* with TT for 4 days, and responding cells were cloned at one cell per well in the presence of autologous feeder cells and TT. Clones were expanded with autologous feeder cells plus TT during the initial 4 weeks and were subsequently propagated with interleukin-2 (IL-2)-enriched T-cell growth factor preparations. This procedure was adopted to obviate outgrowth of nonspecific cells induced by exogenous IL-2 during the cloning procedure. Clones were reselected every 14-21 days with irradiated feeders and TT in the absence of exogenous IL-2. Several stable clones were isolated and screened for major histocompatibility complex (MHC) restricted antigen recognition using a 3-day *in vitro* proliferation assay in the presence of irradiated APC and antigen¹⁰.

We identified several clones recognizing TT in the context of conventional HLA-DR antigens (1C1, 2C3, 3B11), and also several anomalous clones, one of which had apparent allo-reactivity for histocompatibility antigens, as well as specific reactivity for TT + 'self'-MHC. We confirmed the dual specificity of this latter clone (1C1, see below). Detailed functional characterization of these clones will be presented elsewhere (E.N.-B. *et al.*, in preparation).

Intracellular free calcium levels were measured using the

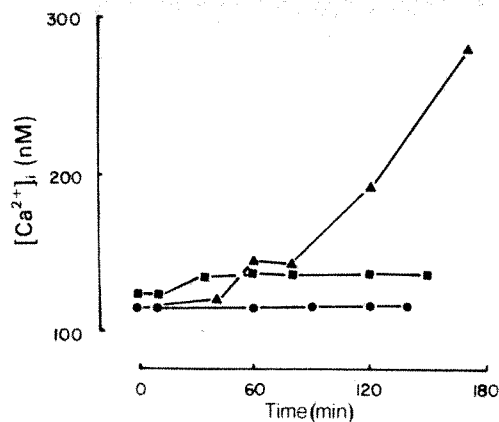


Fig. 1 Cytoplasmic free calcium concentrations ($[Ca^{2+}]_i$) in cloned human T cells after specific activation by antigen. Cells of clone 1C3 (HLA-DR7 restricted) were loaded with Quin-2, and $[Ca^{2+}]_i$ measured at intervals after the following additions: ●, none; ■, autologous HLA-DR2, 7 APC; ▲, autologous APC + TT (0.5 LF ml $^{-1}$). Cloned cells show a progressive rise in $[Ca^{2+}]_i$ only in the presence of antigen and APC.

Methods. T-cell clones: PBM were isolated from a TT-immune HLA-DR2,7 donor by centrifugation on Ficoll-Hypaque, and restimulated *in vitro* for 4 days. Cultures were collected and cells from the largest 20% size fraction were cloned at one cell per well with the Autoclone attachment on a Coulter EPICS-V flow cytometer in the presence of autologous irradiated (4,000 R) PBM and 0.5 LF ml $^{-1}$ TT. Wells showing growth were fed weekly for 3 weeks with fresh irradiated APC and antigen, and subsequently fed twice weekly with a mixture of 10% of a culture supernatant from the MLA-144 cell line²² and 10% of a PHA-induced PBM culture supernatant²³. Every 2–3 weeks, clones were reselected with fresh autologous APC and antigen. Cloned cells were screened for antigen specificity with a 3-day *in vitro* proliferation assay against irradiated PBM from members of a panel of HLA-DR typed donors and 0.5 LF ml $^{-1}$ TT⁸. Proliferative and $[Ca^{2+}]_i$ responses were maximized if cloned cells were deprived of IL-2 in culture for 24–48 h before assay. A similar finding has been reported in mice²⁴. The proliferative responses of cloned T cells were compared with and without Quin-2/AM loading (5 μ M). Comparable stimulation indices (25–50) were observed despite the presence or absence of Quin-2 from the responding cells. Cytoplasmic free calcium determinations: Cloned cells were loaded with Quin-2 by incubation of a suspension of 1×10^7 cells ml $^{-1}$ in RPMI-1640 medium + 10% fetal calf serum (FCS) with 5 μ M Quin-2/AM for 60 min at 37 °C, then washed and resuspended at 1×10^7 cells ml $^{-1}$ (ref. 14). Antigen-presenting cells (4,000 R irradiated PBM) were added at 1×10^7 cells ml $^{-1}$ and TT at a final concentration of 0.5 LF ml $^{-1}$ where indicated. For measurement of fluorescence, aliquots of this suspension were diluted 1:20 in HEPES-buffered phosphate-buffered saline containing 1 mM Ca^{2+} (ref. 14). Quin-2 fluorescence emission was measured at 492 nm with excitation at 395 nm. After each measurement, 100% saturation of the Quin-2/ Ca^{2+} complex was determined in the presence of 1 μ M ionomycin. Cell auto-fluorescence was measured after addition of 0.5 mM Mn^{2+} , which quenches fluorescence of the Quin-2/ Ca^{2+} complex. $[Ca^{2+}]_i$ was calculated from % Ca^{2+} /Quin-2 saturation⁶. Results could not be accounted for by simple leakage of Quin-2 from damaged cells. Quin-2 fluorescence emission in the supernatant fluid after removal of cells by centrifugation was minimal and comparable in all groups, whether stimulated by APC + antigen or unstimulated.

trapped fluorescent indicator Quin-2, which binds Ca^{2+} with 1:1 stoichiometry and shows markedly enhanced fluorescence on binding calcium¹⁶. Cells were loaded with Quin-2 by preincubation with its membrane-permeant acetoxymethyl ester (Quin-2/AM), which undergoes intracellular hydrolysis to the impermeant form¹⁷. The concentration of Quin-2/AM used (5 μ M) in preincubation was not high enough to cause concentration-dependent direct metabolic and mitogenic effects^{4,7}. $[Ca^{2+}]_i$ was calculated as described previously^{4,6}. In preliminary experiments to determine the magnitude and time course of the $[Ca^{2+}]_i$ response, Quin-2-loaded cells of clone 1C3 (HLA-DR2, 7; HLA-DR7-restricted) were mixed with autologous irradiated PBM

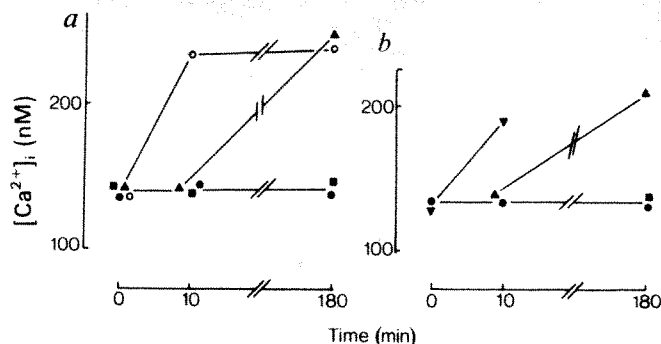


Fig. 2 *a*, Kinetics of the $[Ca^{2+}]_i$ response of clone 2C3 (HLA-DR2 restricted) to APC-associated antigen. Assay was carried out as described in Fig. 1 legend. $[Ca^{2+}]_i$ was determined 10 and 180 min after the following additions at $t=0$: ●, none; ■, HLA-DR2, 7 (autologous) APC; ▲, HLA-DR2, 7 APC + 0.5 LF ml $^{-1}$ TT; ○, HLA-DR2, 7 APC pulsed for 3 h with 0.5 LF ml $^{-1}$ TT, and washed extensively. *b*, Kinetics of the $[Ca^{2+}]_i$ response of cells from 1C1, a T-cell clone with dual specificity for HLA-DR7 + TT and for a non-HLA-DR alloantigen present on an HLA-DR7, 8 cell. $[Ca^{2+}]_i$ was determined at $t=0$, 10 min and 180 min after the following additions: ●, none; ■, HLA-DR2, 7 (autologous) APC; ▲, HLA-DR2, 7 APC + 0.5 LF ml $^{-1}$ TT; ▼, HLA-DR7, 8 stimulator cells.

(as a source of antigen-presenting cells) in the presence or absence of TT. Changes in fluorescence intensity in the cloned cells were followed over time. Possible changes in $[Ca^{2+}]_i$ in the antigen-presenting cells do not interfere with these measurements, as the APC are not labelled with Quin-2. Cloned cells in the presence of autologous feeder cells and TT showed a rise in $[Ca^{2+}]_i$ over a period of 2–3 h, from a basal level of 115 nM to a maximum of 280 nM (Fig. 1). This slow response contrasts with those to phytohaemagglutinin (PHA) or concanavalin A (Con A), in which increased $[Ca^{2+}]_i$ occurs within 2–3 min^{5–7}, but is comparable with that observed following addition of *Staphylococcus* protein A (ref. 14). No increase in $[Ca^{2+}]_i$ was observed in cells of this clone in the presence of TT or of feeders alone. Several other clones were screened using this technique (Table 1). The antigen specificity and MHC restriction of these clones, as reflected in the $[Ca^{2+}]_i$ response, were in all instances concordant with that from 3-day *in vitro* proliferation studies, measured by ^3H -thymidine incorporation. Autologous feeder cells incubated overnight with TT and then washed free of excess antigen induced overall increases in $[Ca^{2+}]_i$, comparable to those seen with autologous feeders plus soluble TT, although the time course of the response was different (see below) (Table 1, clones 1C3 and 3B11).

The 2–3-h latency of the $[Ca^{2+}]_i$ response to soluble antigens and certain mitogens such as *Staphylococcus* protein A may reflect the need for time-dependent antigen 'processing' by accessory cells for efficient antigen presentation and recognition. The latent period should therefore be markedly reduced if the responding T cells are exposed to processed, APC-associated antigen^{12,13}. We tested this prediction for clone 2C3 (Fig. 2*a*). Autologous feeders pulsed with TT for 3 h at 37 °C and then washed extensively induced a rapid (<10 min) increase in $[Ca^{2+}]_i$ from 141 to 235 nM in the responding T cells. Cloned cells in the presence of unpulsed feeders and soluble TT showed no increase in $[Ca^{2+}]_i$ between 0 and 10 min, although $[Ca^{2+}]_i$ did rise slowly to 250 nM over 2 h in these conditions, confirming our earlier findings. Similar results have been reported for protein A, which requires monocyte-macrophage processing to induce $[Ca^{2+}]_i$ and proliferative responses¹⁴. Antigen processing or presentation is rate limiting for the $[Ca^{2+}]_i$ response, and sequential measurement of $[Ca^{2+}]_i$ thus provides a rapid and sensitive means of following events in antigen processing.

Finally, we identified several T-cell clones expressing apparent dual specificity for TT + self-MHC, and for allogeneic MHC. Clones of this type have been described previously in

Table 1 Cytoplasmic calcium concentrations in T-cell clones specifically activated by antigen

Clone	$[Ca^{2+}]_i$ (nM),				
	$t = 0$	$t = 2$ h	$t = 2$ h	$t = 2$ h	$t = 2$ h
	+0	+DR2, 7*	+DR2, 7 + TT	+DR7, 8*	+DR7, 8 + TT
1C1	135	138 (<1)	210 (5)†	ND	ND
1C3	115	135 (<1)	280 (44)†	ND	ND
			244‡		
2C3	135	146 (1)	253 (47)†	152 (1)	153 (1)†
3B11	123	147 (1)	285 (11)†	142 (1)	150 (1)†
			198‡		146‡
3D6§	134	142 (<1)	161 (<1)†	142 (<1)	275 (7.4)†

Cytoplasmic calcium concentrations were determined as described in Fig. 1 legend. $[Ca^{2+}]_i$ was determined at $t = 0$ and $t = 2$ h. Values in parentheses indicate stimulation index obtained in a 3-day *in vitro* proliferation assay. ND, not done.

* APC (4,000 R irradiated PBM) of the indicated HLA-DR type added at $t = 0$.

† APC (4,000 R irradiated PBM) of the indicated HLA-DR type + 0.5 flocculation units (LF) per ml soluble TT added at $t = 0$.

‡ APC of the indicated HLA-DR type, incubated for 3 h at 37 °C with 0.5 LF ml⁻¹ TT and washed free of excess antigen, added at $t = 0$.

§ Clone 3D6 recognizes TT in the context of a private HLA-DR specificity on HLA-DR 7, 8 APC (E.N.-B. *et al.*, in preparation).

the mouse¹⁸, but not in humans. One such clone, 1C1, proliferated in response to soluble TT and autologous HLA-DR2, 7 PBM, and also to non-HLA-DR antigens on a semi-allogeneic HLA-DR7, 8 stimulator cell. This was also seen in the $[Ca^{2+}]_i$ response: autologous PBM plus soluble TT stimulated a $[Ca^{2+}]_i$ response of 2 h latency, while semi-allogeneic stimulator cells alone induced a rapid increase in $[Ca^{2+}]_i$ (Fig. 2b; compare with Fig. 2a). Although these studies do not elucidate the mechanism of alloantigen recognition by this clone, the time course of activation implies that alloantigen is seen directly¹⁹, rather than in association with self-MHC after processing²⁰.

These results suggest that the changes in $[Ca^{2+}]_i$ seen following antigen-specific activation of lymphocytes are similar to those in cells activated by nonspecific mitogens. There is, however, a lag phase in the $[Ca^{2+}]_i$ response to antigen that is compatible with antigen processing. It is unclear if changes in $[Ca^{2+}]_i$ following exposure to mitogens are involved in the control of lymphocyte proliferation and differentiation or occur secondarily. Mitogenic lectins such as PHA and Con A can bring about increases in $[Ca^{2+}]_i$ in conditions in which a proliferative response is not obtained, as can the non-mitogenic lectin wheat germ agglutinin (WGA)⁶. Conversely, proliferative responses can be obtained in the virtual absence of extracellular Ca^{2+} , in cells exposed to PHA and co-mitogens such as phorbol esters²¹. The fact that levels of $[Ca^{2+}]_i$ increase rapidly in response to appropriate antigenic stimulation, and correlate directly with cell activation leading to proliferation *in vitro*, supports a role for increased $[Ca^{2+}]_i$ as an intracellular messenger in activation.

This technique provides a new and sensitive method to study early events in lymphocyte activation by antigens, and also to address the cellular mechanisms involved in antigen processing and presentation⁷.

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Stimulated neutrophils from patients with autosomal recessive chronic granulomatous disease fail to phosphorylate a M_r 44,000 protein

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Phagocytosing neutrophils, monocytes, macrophages and eosinophils produce a burst of non-mitochondrial respiration that is important for the killing and digestion of microbes. Much of the information about the oxidase system involved comes from studies on patients with chronic granulomatous disease (CGD), a syndrome in which an undue predisposition to infection results from complete absence of this burst of stimulated respiratory activity¹. The basis of the oxidase activity is an electron transport chain, the only established component of which is a very unusual *b*-type cytochrome (*b*₂₄₅) (ref. 2). The molecular defect in the X-linked subgroup of CGD is the absence of this cytochrome *b*₂₄₅, which, however, appears to be normal in those subjects with the autosomal recessive mode of inheritance³. In an attempt to identify an abnormality of activation, or an absence or malfunction of a proximal component of the electron transport chain in this latter group, we examined protein phosphorylation in neutrophils after activation of the oxidase with phorbol myristate acetate. All four of the patients studied demonstrated a selective lack of the enhanced phosphorylation of a protein of relative molecular mass (M_r) 44,000 (44K) that was observed in normal subjects and in two CGD patients with an X-linked inheritance. This molecule, therefore, could be an important functional component of the oxidase.

Neutrophils exposed to soluble and particulate activators of the oxidase, including phorbol myristate acetate (PMA), which is known to activate protein kinase C directly⁴, have been shown to rapidly phosphorylate a number of proteins⁵⁻⁷. We studied the time course and dose response of phosphorylation of human neutrophil proteins in intact cells stimulated with PMA. Figure 1 shows that many proteins become phosphorylated in ostensibly resting cells, and that at least 10 of these, including some with apparent M_r s similar to those described previously⁵⁻⁷, undergo enhanced phosphorylation on stimulation. The most marked incorporation occurred in bands with M_r s of 40, 44, 47, 50 and 64K and a broad band between 70 and 90K. This activated phosphorylation occurred rapidly, with obvious changes after 5 s, and continued to increase for at least 6 min.

The rate of increase in ³²P-incorporation was not uniform

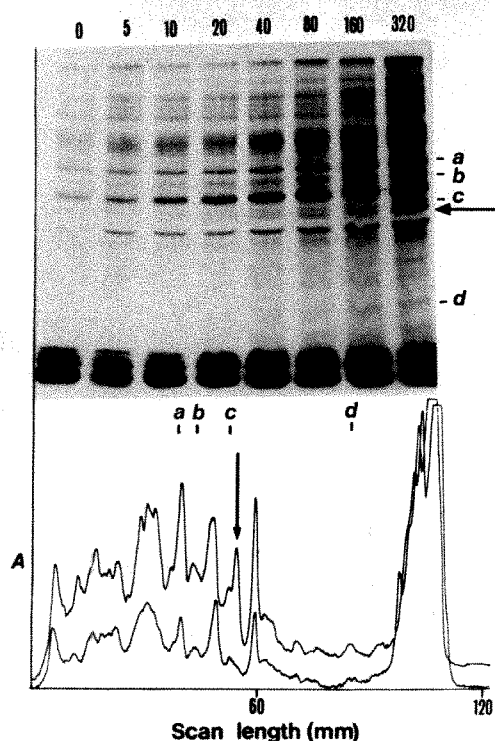


Fig. 1 Protein phosphorylation in normal neutrophils at varying times after stimulation with PMA. *a-d* Represent the distances migrated by M_r markers: *a*, 77K; *b*, 66.7K; *c*, 45K; *d*, 25.7K, and the arrow indicates the position of the 44K phosphorylated protein. **Methods.** Cells (4×10^7) were purified from peripheral blood¹¹, washed in Tyrode's solution¹⁹ containing bovine serum albumin (0.025% w/v), then incubated for 1 h at 37 °C in 1 ml of the same solution containing 1 mCi ^{32} P (Amersham International, PBS.11); 10 μ g of PMA in 10 μ l dimethyl sulphoxide (DMSO) were added and 100- μ l aliquots taken at timed intervals (0-320 s) into 1 ml of cold 10% trichloroacetic acid. After 1 h the precipitates were pelleted at 12,000 r.p.m. at 4 °C for 3 min, then washed twice in Na phosphate (50 mM, pH 7.4)-buffered saline. The pelleted proteins were solubilized in SDS sample buffer and separated by SDS-polyacrylamide gel electrophoresis on 10% polyacrylamide slab gels using the Laemmli system of buffers²⁰. The dried gels were autoradiographed against Fuji-Rx film for 3 days (top). The zero time track (lower trace at bottom of figure) and the 320-s track (upper trace) were scanned with a Joyce-Loebl Chromo-scan 3.

throughout all the bands, being very rapid in some—for example, that at ~40K—and slowly progressive in others, particularly that with a M_r of ~44K. Phosphorylation was clearly dependent on concentration of PMA. Stimulation was detected at 1 ng ml⁻¹, reached a plateau at 100 ng ml⁻¹ and remained constant thereafter to a concentration of 100 μ g ml⁻¹. We chose standardized conditions for the stimulation of 10^7 cells, that is, 10 μ g PMA ml⁻¹ for 1 min and 10 min for most of the subsequent studies.

Eight normal subjects were examined and a consistent pattern of stimulated phosphorylation observed. We then examined the pattern of phosphorylation in four subjects with the very rare autosomal recessive form of CGD. Two of these were siblings from Uppsala in Sweden while the other two were English women (one was patient no. 25 in ref. 3). Cells from all four patients showed a specific absence of the normal enhancement of the phosphorylation of a protein with an apparent M_r of 44.0 ± 0.99 K (mean $\pm$ s.d. of 21 electrophoresis tracks from eight subjects: Figs 2, 3). The protein bands in this region of the gel, which were stained with Coomassie blue, appeared normal. Faint bands of phosphorylation were sometimes observed in this part of the gel in unstimulated cells from both these patients and controls; however, the normal stimulated increase in phosphorylation was never observed in the patients (Fig. 3). Apart from the abnormality in this band, the pattern of protein phos-

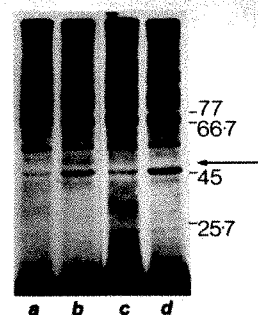


Fig. 2 Protein phosphorylation in control (*a, b*) and autosomal recessive CGD (*c, d*) neutrophils. Cells were prepared as described in Fig. 1 legend and treated for 10 min with 10 μ l DMSO (*a, c*) or an equal volume of DMSO containing 10 μ g PMA (*b, d*). Subsequent procedures were as described for Fig. 1. The protein of specific interest (arrow) demonstrates enhanced phosphorylation in the normal subject but not in the patient. The distances migrated by the M_r markers are indicated.

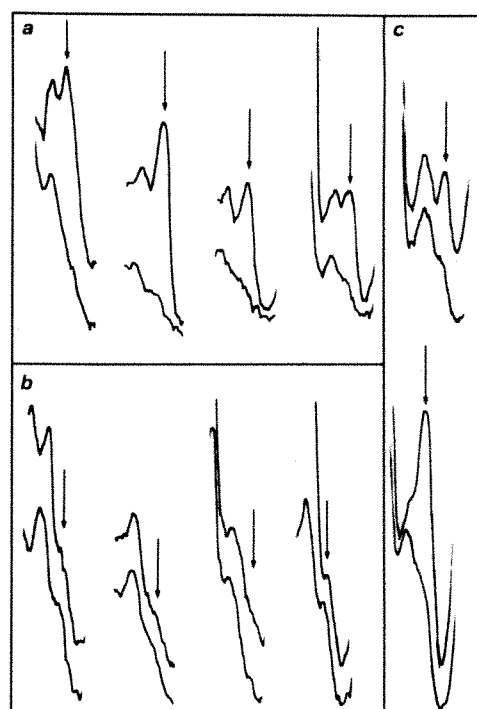


Fig. 3 Densitometry scans of autoradiographs in the region of the 44K protein. In each case the lower scan was performed on the track from unstimulated cells, and the upper scan on the track from stimulated cells. The scans were then superimposed to facilitate comparison of the same region of the gel. The 44K phosphoprotein is indicated by the arrows. Enhanced phosphorylation of this band is not observed in cells from the patients with CGD inherited in an autosomal recessive manner (*b*), whereas it is seen in cells from their corresponding control subjects (*a*) and in two patients with an X-linked pattern of inheritance (*c*).

phorylation was normal in cells from these patients.

To ensure that the absence of enhanced phosphorylation of the 44K protein band in these patients was specific for this subgroup of the syndrome and not a consequence of the failure of the oxidase system as a whole, two patients with classical X-linked CGD were studied. An entirely normal pattern of phosphorylation was observed after activation of the cells from both patients (Fig. 4): an English man (patient 4 in ref. 3) and a Greek Cypriot boy, whose cells lack cytochrome *b*₂₄₅ (ref. 3).

To quantitate these changes, the autoradiographs were scanned with a Joyce Loebl Chromoscan 3 gel scanner (slit width of 0.1 mm at an absorbance of 1.0). None of the patients with the autosomal recessive variant of the syndrome demonstrated any increase in absorbance in the region of the 44K band (limit of detection ~1 mm), whereas their paired control subjects

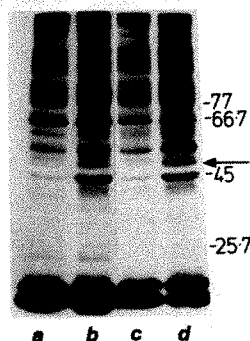


Fig. 4 Protein phosphorylation in control (a, b) and X-linked CGD (c, d) neutrophils. Cells were prepared and treated as described in Fig. 1 legend. a, c, Cells treated with DMSO alone; b, d, cells treated with DMSO and PMA. The distances migrated by the M_r markers are indicated, and the arrow shows the position of the band of phosphorylation of specific interest.

showed a rise in peak height of 15.3 ± 2.8 mm (mean $\pm$ s.d.) and in the X-linked patients these values were 22.9 and 12.1 mm (Fig. 3). In comparison, another band with an apparent M_r of 40K that generally becomes heavily phosphorylated (Fig. 1) showed a normal enhancement in the patients. The increase in this peak of absorption was 75.5 ± 32.1 mm in the healthy subjects, 81.6 ± 30.1 mm in the autosomal patients and 94 and 66 mm in the X-linked patients.

The subcellular distribution of this 44K phosphorylated protein was determined in stimulated and unstimulated neutrophils fractionated on discontinuous sucrose gradients⁸. The enhancement of phosphorylation after stimulation appeared to be roughly equally distributed between the membrane and granule fractions, with little activity in the cytosol. This distribution corresponds with that of cytochrome *b*, as would be expected if the two molecules interact. Phagocytosis of latex particles opsonized with human IgG also resulted in the enhanced phosphorylation of this 44K band, which was detected in association with purified phagocytic vacuoles⁹ (data not shown).

The only other study of protein phosphorylation in CGD that we have found is that by Andrews and Babior⁶ in which they investigated three patients, two males and one female, using basically similar techniques to those used in the present study. However, although the female patient, at least, probably had the autosomal recessive variant of the disease, they failed to observe any abnormalities of phosphorylation in these patients. This discrepancy could be a reflection of the heterogeneity¹⁰ of the syndrome or a minor technical difference.

After we identified the electron transport chain and its very unusual cytochrome *b*₂₄₅, we then examined its composition and integrity in CGD, the condition resulting from an absence of the oxidative burst. In a large study the cytochrome was shown to be missing from the cells of all patients with the X-linked condition, but present and ostensibly normal in those patients with the autosomal recessive mode of inheritance^{3,11}. The cytochrome, however, does not receive electrons on stimulation of the cells with an activator of the oxidase, a situation compatible with the absence of a proximal electron donor or a defect of activation^{3,11}.

Other components of the chain have not been identified, but an FAD-containing flavoprotein has been implicated since the earliest studies on the 'enzyme' system¹²⁻¹⁷. However, the flavoprotein component of the oxidase has not been clearly defined or shown to be abnormal in CGD, largely because these proteins are more difficult to study than cytochrome *b* because of their lower extinction coefficient, broad spectrum of light absorbance, the ease with which the flavin dissociates from the apoprotein and the high abundance of these proteins in neutrophils.

As shown here, there is clearly a specific defect of phosphorylation in the cells of patients with autosomal recessive CGD, an abnormality that provides the first real lead to the molecular

lesion in these subjects. In view of the different genetic pools of the patients this abnormality is unlikely to result from the expression of a gene linkage that is unrelated to the oxidase. Normal phosphorylation in the X-linked condition indicates that it is unrelated to the absence of oxidase activity. It could be that this protein is a proximal electron-transporting molecule in the oxidase chain or some other essential component, and that it fails to become activated because of defective phosphorylation. This is more likely to be due to an abnormality of the protein, possibly an amino-acid transposition, with substitution of serine or threonine, the normal targets of phosphorylation¹⁸, than a defect of the phosphorylating system, as other proteins show enhanced phosphorylation in their stimulated neutrophils. The band of phosphorylation observed in the same region of the gels in resting cells from these patients could indicate a different protein or phosphorylation of sites on the same protein that are not associated with activation by PMA.

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Human γ -chain genes are rearranged in leukaemic T cells and map to the short arm of chromosome 7

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Three gene families that rearrange during the somatic development of T cells have been identified in the murine genome. Two of these gene families (α and β) encode subunits of the antigen-specific T-cell receptor and are also present in the human genome¹⁻⁵. The third gene family, designated here as the γ -chain gene family, is rearranged in murine cytolytic T cells but not in most helper T cells⁶⁻⁸. Here we present evidence that the human genome also contains γ -chain genes that undergo somatic rearrangement in leukaemia-derived T cells. Murine γ -chain genes appear to be encoded in gene segments that are analogous to the immunoglobulin gene variable, constant and joining segments⁴. There are two closely related constant-region gene segments in the human genome. One of the constant-region genes is deleted in all three T-cell leukaemias that we have studied. The two constant-region γ -chain genes reside on the short arm of chromosome 7

Fig. 1 Restriction map and nucleotide sequence of the human $C_{\gamma}1$ constant-region gene segment. **a**, The $C_{\gamma}1$ constant-region segment lies on a 5.0-kb *EcoRI* restriction fragment. The location of the constant-region coding block (cross-hatched region) was determined by hybridization with a mouse γ cDNA probe. The locations of the *EcoRI* (R), *HindIII* (H), *PstI* (P) and *BamHI* (B) restriction enzyme sites are indicated. The nucleotide sequence of the coding block was determined by dideoxynucleotide sequencing using M13 cloned fragments and sequencing in the directions shown by the arrows. **b**, Nucleotide sequence of the human $C_{\gamma}1$ constant-region fragment (hum) compared with the mouse γ constant-region segment (mou). The predicted amino-acid sequence (hum aa) and the differences between the human and the mouse proteins (asterisks) are indicated above the nucleotide sequence of the human gene.

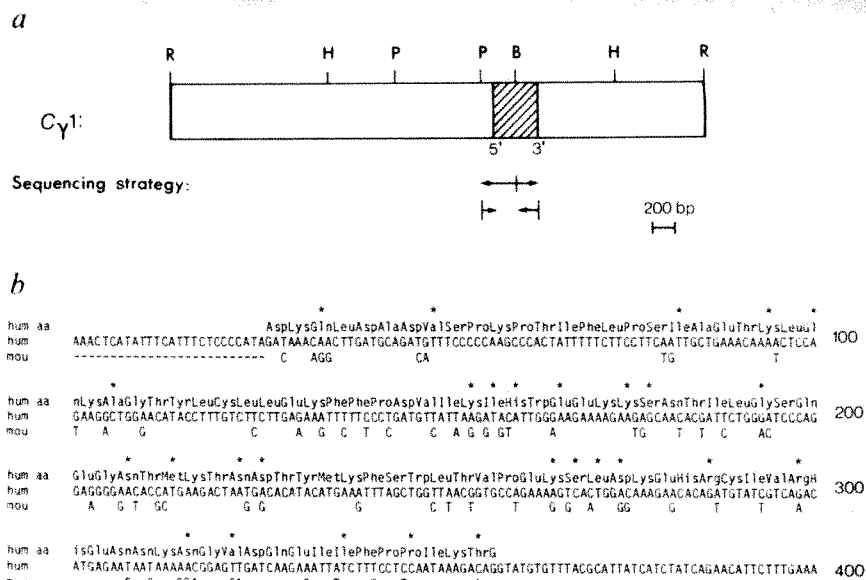
Methods. **a**, A cDNA library was constructed from MD26 (a murine cytolytic T cell) poly(A)⁺ mRNA^{18,19} as described previously²⁰. The library was screened with a 14-base oligonucleotide (ACTGTGCAGTCTGG, provided by Ellen K. Doran) as described previously²⁵. The nucleotide sequence of the isolated cDNA was identical to the cDNA isolated by Saito *et al.* (ref. 6 and data not shown). Human B-cell lymphoma (U266) DNA was digested with *EcoRI*, and the 5.0- and 7.2-kb fragments that hybridized with the murine cDNA probe were cloned into pBR322 (ref. 20 and see text also).

(7p15); this region is involved in chromosomal rearrangements identified in T cells from individuals with the immunodeficiency syndrome ataxia telangiectasia⁹⁻¹² and observed only rarely in routine cytogenetic analyses of normal individuals¹³⁻¹⁶. This region is also a secondary site of β -chain gene hybridization¹⁷.

Segments encoding human γ -chain constant-region genes were cloned using the murine complementary DNA probe (see legend to Fig. 1a; C.M. and J.G.S., unpublished results). Southern blots of human DNA using a γ -chain probe derived from the murine T cell MD26 (refs 18, 19) revealed two weakly hybridizing bands (data not shown). Two *EcoRI* fragments of 5.0 and 7.2 kilobases (kb) were partially purified from digests of human peripheral blood DNA by fractionation on a preparative agarose gel²⁰. Subgenomic libraries were constructed from these partially purified DNA fragments by cloning into the bacterial plasmid pBR322. Three independent plasmids encoding the 5.0-kb fragment were isolated from ~200,000 plasmids containing the size-fractionated genomic DNA. The subgenomic libraries were screened with the murine γ -chain cDNA probe. Figure 1a shows a partial restriction map of the 5.0-kb fragment and the location of the γ -chain exon within it.

The nucleotide sequence of the constant-region portion of the γ gene encoded by the 5.0-kb *EcoRI* fragment was determined (Fig. 1). The 5.0-kb fragment contains only a single exon of the human γ gene and this segment is highly homologous to the murine γ gene constant-region segment (Fig. 1b). This segment is bounded by sequences that are characteristic of RNA splicing signals and thus could be a functional coding block²¹. The human and murine constant-region gene segments are identical at 274 of 330 base pairs (bp) (83% homology). The human gene segment would encode a protein that is 75% homologous to the putative murine polypeptide.

To determine the number of γ constant-region genes that exist in the human genome and to determine whether these genes are rearranged in the human leukaemia-derived T cells, we have analysed Southern blots of human B- and T-cell DNA. Southern blots of peripheral blood lymphocyte DNA obtained from two patients with mature T-cell leukaemia and from one patient with an immature T-cell leukaemia, as well as B-cell lines from the same individuals, were probed with a 300-bp *PstI/Bam* fragment (Fig. 1a) that encodes part of the constant-region segment and its 5' flanking sequence. Only two strongly hybridizing bands were identified in *EcoRI*, *HindIII*, *PstI*, *SstI*, *BamHI* and *PvuII* digests of human B-cell DNA (Fig. 2, lanes



B1, B2 and B3, and our unpublished results). Because both *EcoRI* bands were identified in the Southern blots of DNA from 20 different individuals (C.M. and J.G.S., unpublished data), we believe that these two fragments encode non-allelic constant-region genes. We suggest that there are only two constant-region segments that are more than 80% homologous to the murine γ -chain constant-region segments, although other, more distantly related constant-region genes may exist.

If the γ genes of human T cells undergo somatic rearrangement, we would expect these constant region-containing gene fragments to be altered in human T-cell DNA. The DNAs from two mature T-cell leukaemias (Fig. 2, lanes T1 and T2) and from one immature T-cell leukaemic line (Fig. 2, lane T3) were analysed by Southern blotting. The 5.0-kb *EcoRI* and 2.8-kb *HindIII* fragments are deleted in the leukaemic T cells. The small amounts of these fragments seen in lanes T1, T2 and T3 of Fig. 2 are contaminating peripheral blood T cells. These differences do not result from polymorphic differences between the different

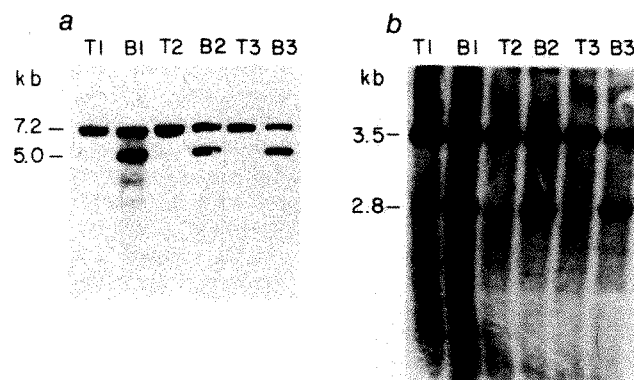


Fig. 2 Southern blots of human B- and T-cell DNAs probed with a human C_{γ} probe (*PstI/BamHI* in Fig. 1a). DNAs from two mature adult T-cell leukaemic lines (Falc (T1) and Sylv (T2)), one immature T-cell leukaemic 8402T (T3), and B-cell lines from the same individuals, referred to as B1 (CF2K), B2 (HLS) and B3 (RPMI 8392), were digested with *EcoRI* (a) and *HindIII* (b) fractionated on agarose gels, transferred to nitrocellulose filters and hybridized using methods described previously²⁰. The sizes of the hybridizing fragments were determined by co-migration with bacteriophage λ *HindIII* fragments. kb, kilobases.

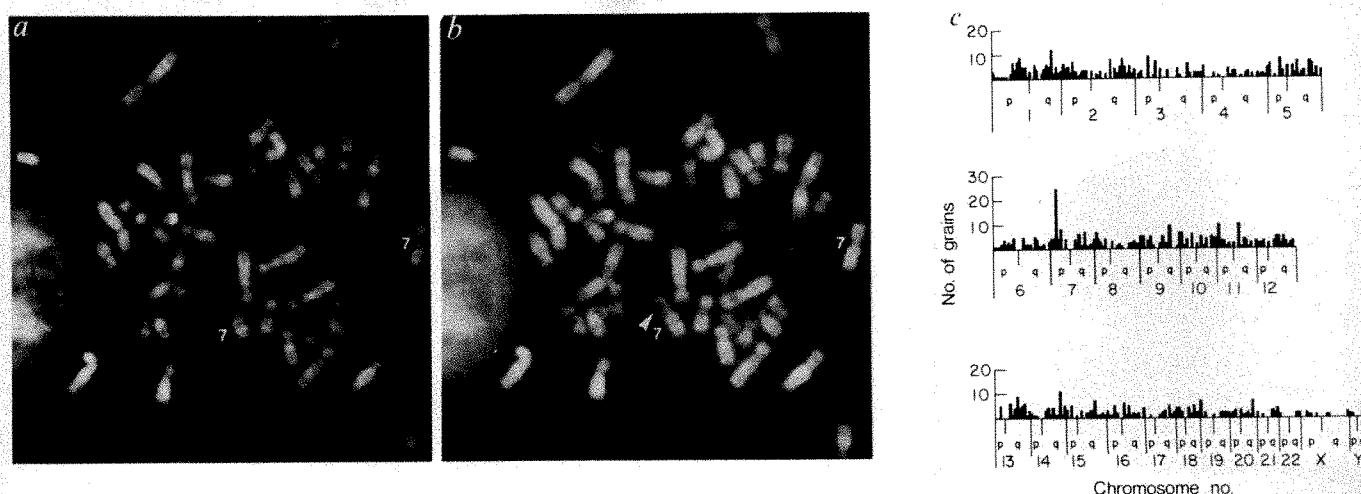


Fig. 3 The γ -chain constant-region locus is assigned to the short arm of chromosome 7 at band 7p15 by chromosomal *in situ* hybridization. *a*, Photograph of metaphase chromosome spread from a normal human male, stained with quinacrine mustard dihydrochloride and visualized by incident ultraviolet light. *b*, Photograph of the same metaphase spread as in *a*, visualized by a combination of incident ultraviolet and transmitted visible light. A grain can be seen on the short arm of one chromosome 7 in the region of 7p15. *c*, Distribution of silver grains for 233 metaphase spreads derived from PHA-stimulated peripheral blood lymphocytes following hybridization with a $C_{\gamma}1$ -derived probe (the *PstI/BamHI* fragment indicated in Fig. 1). The histogram corresponds to the standard chromosome idiogram²⁹. Significant hybridization is seen on the short arm of chromosome 7 in band p15; 10% of all metaphases contained a grain on 7p15.

Methods. Metaphase chromosomes were prepared from PHA-stimulated peripheral blood lymphocyte cultures established from two normal males according to standard cytogenetic protocols³⁰. A subclone containing the *PstI/BamHI* fragment (Fig. 1*a*) was labelled with tritium by nick-translation as described previously³¹. *In situ* hybridization, autoradiography, and chromosome banding and analysis were performed as reported elsewhere³¹.

individuals from which the T-cell leukaemic lines were isolated, as the B-cell lines were isolated from the same individuals. Thus, these T-cell leukaemias appear to have undergone a specific deletion of one constant-region segment. Two models can be proposed to account for the deletions of these constant-region gene segments: one possibility is that the gene rearrangements are related to translocations occurring during the somatic development of the T cells; a more likely possibility is that these deletions occur during the somatic formation of an active γ -chain gene. Either these cells have undergone this recombination on both chromosomes, or one of the chromosomes encoding the γ -chain gene locus has been lost from these T cells, as has been reported in T cells derived from some patients with aplastic anaemia²².

We needed to localize the γ -chain gene locus on the chromosome to determine whether chromosomal rearrangements occur at or near it. Because the β -chain genes appear to hybridize with the germline locus of the β -chain genes and with a secondary site¹⁷ in human peripheral blood lymphocytes, we used two independent approaches to map the γ -chain gene locus. First, we used *in situ* hybridization of a γ -chain probe to metaphase chromosomes derived from phytohaemagglutinin (PHA)-stimulated human peripheral blood cells (Fig. 3). Analysis of 233 metaphase spreads obtained from two normal individuals revealed that ~2.6% of the silver grains were located on or adjacent to chromosome 7 at band 7p15. The 300-bp *PstI/BamHI* hybridization probe was chosen for these experiments because several other fragments derived from the cloned 5.0-kb *EcoRI* fragment contained reiterated sequences. Secondary peaks of grain accumulation were noted at 1q32 (1.3%), 11p15 (1.1%), 11q12 (1.1%) and 14q24 (1.2%). The significance of these secondary peaks of hybridization remains to be elucidated.

The second approach to mapping the γ -chain locus involved the use of human/mouse somatic cell hybrids. The constant-region probe showed hybridization to *SstI*-digested genomic DNA derived from a hybrid cell line containing only human chromosome 7 and a hybrid cell line containing several human chromosomes and a portion of human chromosome 7 including the short arm (refs 23, 24 and T.B.S., J.G.S. and C.M., data not shown). Thus, two independently derived hybrid cells containing human chromosome 7 also contain the γ -chain locus.

The assignment of the γ -chain gene locus to 7p15 is of note for two reasons. Interestingly, earlier studies demonstrated that human T-cell β -chain gene probes hybridize to this chromosomal band in addition to the primary assignment at 7q32 (refs 17, 25). We now question whether the secondary peak of hybridization is related to the presence of the γ -chain locus at 7p15. However, we note that the secondary peak is not a result of direct cross-hybridization between the β - and γ -chain genes as the cloned segments do not cross-hybridize (A. Duby, C.M. and J.G.S., unpublished). Alternative explanations for the weak hybridization of the β -chain gene probes to the short arm of chromosome 7 include the presence of another gene family more closely related to β -chain genes than to γ -chain genes or a form of somatic recombination occurring in T cells that moves β -chain genes to the γ -chain locus.

Furthermore, previous cytogenetic analyses have revealed two instances of chromosomal rearrangements involving the short arm of chromosome 7. In particular, metaphase preparations from peripheral blood lymphocyte cultures from individuals with the autosomal recessive immunodeficiency disorder ataxia telangiectasia have shown characteristic rearrangements involving 7p14-15, 7q32-35, 14q11-12 and 14q32 (refs 9-12). Similar sites of rearrangement have been noted occasionally in karyotypes of normal individuals also. Each of these loci include genes that rearrange during somatic development of T cells. We have demonstrated here that the 7p14-15 region of rearrangement contains the γ -chain locus; earlier studies mapped the β -chain locus to 7q32 (ref. 17), and the α -chain genes have been mapped to 14q11 (refs 26-28). We now suggest that the α -, β - and γ -chain loci are good sites for chromosomal translocations because they are involved in site-specific DNA rearrangements. Future studies should determine whether these translocations merely reflect accidents that occur during somatic development of T cells, or whether they play an important part in the development of the immune response or in the oncogenic transformation of T cells.

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Identity of tumour necrosis factor and the macrophage-secreted factor cachectin

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In mammals, several well-defined metabolic changes occur during infection, many of which are attributable to products of the reticuloendothelial system¹⁻³. Among these changes, a hypertriglyceridaemic state is frequently evident⁴⁻⁹, resulting from defective triglyceride clearance, caused by systemic suppression of the enzyme lipoprotein lipase (LPL)⁹. We have found previously that macrophages secrete the hormone cachectin, which specifically suppresses LPL activity in cultured adipocytes (3T3-L1 cells)¹⁰⁻¹⁷. When originally purified from RAW 264.7 (mouse macrophage) cells, cachectin was shown to have a *pI* of 4.7, a subunit size of relative molecular mass (*M_r*) 17,000 and to form non-covalent multimers¹⁷. A receptor for cachectin was identified on non-tumorigenic cultured cells and on normal mouse liver membranes¹⁷. A new high-yield purification technique has enabled us to determine further details of the structure of mouse cachectin. We now report that a high degree of homology exists between the N-terminal sequence of mouse cachectin and the N-terminal sequence recently determined for human tumour necrosis factor (TNF)^{18,19}. Purified cachectin also possesses potent TNF activity *in vitro*. These findings suggest that the 'cachectin' and 'TNF' activities of murine macrophage conditioned medium are attributable to a single protein, which modulates the metabolic activities of normal as well as neoplastic cells through interaction with specific high-affinity receptors.

Cachectin bioactivity (LPL suppression) was assayed using 3T3-L1 cells^{20,21} described previously¹⁷. TNF activity was measured using the standard cytotoxicity assay with actinomycin D-treated L-929 cells²².

A 50-fold concentrate of crude cachectin, derived from lipopolysaccharide-stimulated RAW 264.7 cells, was prepared using a PM-10 membrane in a stirred cell (Amicon) as described previously¹⁷. Before removal of the sample from the ultrafiltration cell, octyl glucoside (Sigma) was added to a concentration of 1% (w/v). (Addition of a non-ionic detergent was essential to achieve high recovery in subsequent steps.)

The sample was passed through a 0.22 µm filter, and applied to a Mono Q (high-performance anion exchange) column

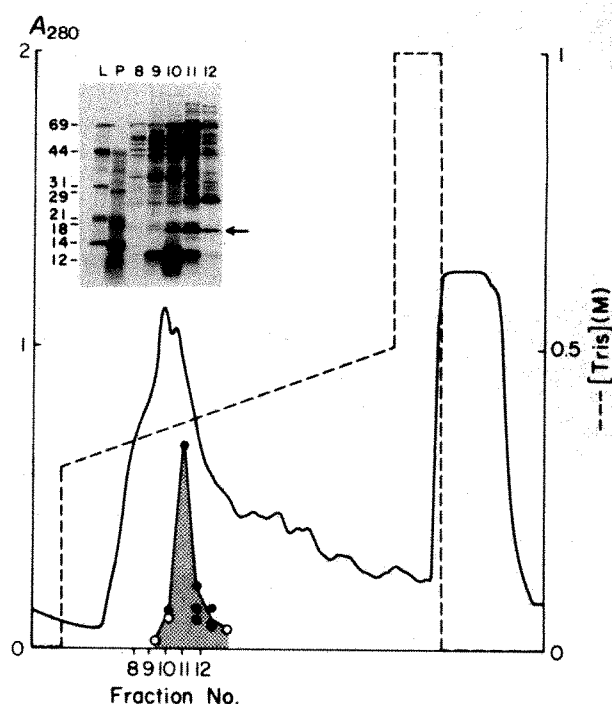


Fig. 1 Mono Q (FPLC anion exchange) chromatography. Crude concentrated RAW 264.7 medium, containing octyl glucoside as a dispersing agent, was applied to the column. Cachectin was eluted using a 0.3 M to 0.5 M Tris-Cl gradient, run at a flow rate 0.33 ml min⁻¹, over 34 min. Absorbance at 280 nm is indicated by the solid tracing. The dashed line indicates the molarity of Tris-Cl buffer used in elution. The cross-hatched area indicates the distribution of cachectin bioactivity, expressed in linear units (each point represents the result of a single bioassay). Inset photograph: a silver-stained²⁷ SDS polyacrylamide gradient gel (10% to 15% acrylamide) showing separation of crude material and successive column fractions (1 µl per lane). Relative molecular mass markers are presented in the far left lanes. The arrow indicates the band corresponding to cachectin (*M_r* 17,000 (17K)). Approximately 60% of the total protein elutes in the non-retained fraction (not shown). The peak of cachectin bioactivity elutes at a concentration of 0.38 M Tris-Cl. The three fractions with highest activity were pooled for further purification.

(FPLC; Pharmacia); ~60% of the total protein passed directly through the column. Cachectin eluted as a sharp peak at a concentration of 0.38 M Tris-Cl, pH 7.8 (Fig. 1); ~80% of the hormone was recovered in three fractions.

These fractions were pooled, lyophilized and redissolved in 0.4 ml distilled water. The sample was then subjected to high-performance gel-filtration chromatography (Superose 12; Pharmacia) (Fig. 2). Cachectin consistently eluted at a position corresponding to an *M_r* of 87,000 consistent with a pentameric

Table 1 Amino-acid composition of cachectin and TNF

	Cachectin	TNF		Cachectin	TNF
Asn/Asp	16	7/5	Leu	17	18
Thr	6	6	Tyr	8	7
Ser	12	13	Phe	6	4
Gln/Glu	20	10/10	His	3	3
Gly	13	11	Lys	10	6
Val	13	13	Arg	5	5
Ala	13	13	Pro	ND	10
Met	2	0	Cys	2	2
Ile	5	8	Trp	ND	2

ND, not determined.

* From the composition established by Pennica *et al.* for human TNF¹⁸.

structure. The molecule readily dissociates in the presence of SDS or 6 M urea, but remains intact in the presence of non-ionic detergents. It is unclear whether a pentameric conformation is essential for bioactivity.

The trailing edge of the cachectin peak was estimated to be >95% pure, and contained approximately 10^7 U cachectin bioactivity per mg protein, as did homogeneous solutions produced by means of the older purification method¹⁷. TNF activity was assayed in this preparation; $\sim 1.2 \times 10^7$ U per mg protein was measured in purified samples, compared with 3.7×10^4 U per mg in a sample of crude material. The specific activity of purified human TNF has been reported to be 2.9×10^7 U per mg protein²³.

The remaining cachectin-rich Superose 12 fractions, to be used in determinations of the amino-acid composition and sequence, were further purified by reverse-phase chromatography, using a C8 column (Supelco) as described previously²⁴. Cachectin eluted at a concentration of 30–40% acetonitrile. Forty pmol of the highly purified protein was hydrolysed with 6 M HCl and used for compositional analysis; 200 pmol was used in sequencing. (A Waters HPLC system, and a microsequencing apparatus (Applied Biosystems model 470 A) were used.)

Comparison of the amino-acid composition of mouse cachectin with that of human TNF (determined by DNA sequencing)¹⁸ reveals strong similarities (Table 1). Both proteins are acidic and hydrophobic, containing identical numbers of threonine, valine, alanine, histidine, arginine and cysteine residues.

N-terminal sequence analysis of mouse cachectin discloses a striking homology with the N-terminal sequence of human TNF (Fig. 3). All but 5 of the first 19 residues are in agreement. These data, considered together with the cytolytic effect of purified cachectin on L-929 cells, suggest that a single protein species is responsible for the cachectin and TNF activity of murine macrophage conditioned medium. Furthermore, this protein seems to be highly conserved.

We have demonstrated previously that cachectin is bound by a specific receptor, present in $\sim 10,000$ copies per cell on cultured 3T3-L1 adipocytes and C2 myotubules¹⁷. The K_d of the hormone-receptor interaction was determined to be 3×10^9 M⁻¹ by Scatchard analysis. Normal mouse liver membranes also bind cachectin in a competitive fashion. A decline in enzymatic activity is observed immediately following addition of the hormone¹¹, and apparently results from the specific inhibition of LPL biosynthesis. Net protein synthesis and cell viability are unimpaired (refs 11, 25 and S. R. Price and P. H. Pekala, manuscript in preparation).

Fig. 3 The N-terminal amino-acid sequence of mouse cachectin, top line, aligned with the corresponding sequence of human TNF¹⁸, bottom line. Equivocal residues are indicated by '?'. Matching residues are underlined.

(Cach) H₂N-leu-arg-ser-ser-ser-gln-asn-ser-ser-asp-? -pro-val-ala-? -
 (TNF) H₂N-val-arg-ser-ser-ser-arg-thr-pro-ser-asp-lys-pro-val-ala-his-
 1 10
 ?
 (Cach) val-val-ala-asn...
 (TNF) val-val-ala-asn...

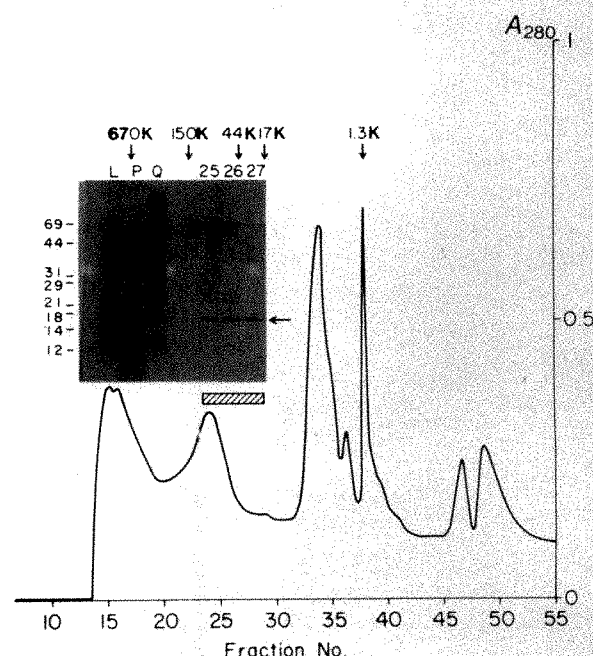


Fig. 2 Superose 12 (FPLC gel filtration) chromatography. Pooled fractions from Mono Q fractionation were lyophilized, redissolved in distilled water, and applied to the column. Chromatography was carried out in 0.1 M ammonium acetate at a flow rate of 0.3 ml min⁻¹. A sample volume of 0.2 ml was used in each separation. The solid tracing represents absorbance at 280 nm. The cross-hatched bar indicates the presence of cachectin, as assessed by measurement of bioactivity. Arrows indicate the elution position of M_r markers applied to the Superose column. Inset photograph: SDS polyacrylamide gradient gel (10% to 15% acrylamide) showing the electrophoretic profile of successive fractions (1 μ l aliquot per lane) derived from Superose 12 gel filtration. L and P, M_r standards; Q, pooled Mono Q fractions (starting material for the Superose 12 separation).

Tumour necrosis factor has previously been studied as a cytolytic agent, capable of killing transformed cells with a high degree of selectivity^{22,26}, after a lag time of 8–16 h. The molecular basis of this selective cytotoxicity has remained elusive. Moreover, the function of the protein *in vivo* has not been established.

However, it is now clear that mouse TNF can bind to receptors present on several normal (non-transformed) murine tissues, and of evoking a specific biochemical response (LPL suppression) in adipocytes¹⁷. As the receptor for TNF is not restricted to adipocytes, we suggest that TNF, when elaborated *in vivo*, elicits specific metabolic responses in various normal host tissues. Moreover, specific suppression of protein biosynthesis might lead to the cytolytic effect observed when certain tumour cells are exposed to the hormone.

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Neurones express high levels of a structurally modified, activated form of pp60^{c-src}

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Neural tissues contain high levels of the cellular homologue of the transforming protein of Rous sarcoma virus (RSV)¹⁻⁴, but neither the specific cell types expressing high levels of c-src, nor the function of the cellular src (c-src) protein has been determined. Using primary culture methods, we have found that pure neurones and astrocytes derived from the rat central nervous system (CNS) contain 15- to 20-times higher levels of the c-src protein than fibroblasts. However, the specific activity of the c-src protein from the neuronal cultures is 6- to 12-times higher than that from the astrocyte cultures. In addition, the c-src protein expressed in neuronal cultures contains a structural alteration within the aminoterminal region of the molecule that causes a shift in the mobility of the c-src protein on the SDS-polyacrylamide gels. These results indicate that a structurally distinct form of the cellular src protein that possesses an activated tyrosylkinase activity is expressed at very high levels in post-mitotic CNS neurones.

Figure 1 shows photomicrographs of the neuronal (A) and astrocytic (B) cultures used in this study. Neuronal cultures were obtained by dissociation of the metencephalon and lower telencephalon from 14-day rat embryos. The cells were cultured in a cerebrospinal fluid-like medium (N5) containing a neurotrophic fraction from horse serum⁵. This serum fraction promotes the maintenance of neurones in culture. The neuronal nature of the majority of cells (>98%) was confirmed by their ability to fire action potentials and bind tetanus toxin⁶ or anti-serum to neurofilaments⁷ (Fig. 1A). The few (<2%) non-neuronal cells were fibrous astrocytes that stained with antibodies to the glial fibrillary acidic protein (GFAP), a specific marker for astrocytes⁸.

Purified astrocytes were prepared from newborn rat cerebral cortices. Cultures were grown in N5 medium supplemented with 5% horse serum. Ninety-nine per cent of these cells displayed

flat-sheet like morphology and stained with antibody to GFAP (Fig. 1B). Other cells (1%) were neurones or fibrous astrocytes, as evidence by binding of tetanus toxin and monoclonal antibody to A2B5⁹.

Lysates from these cultured cells were assayed for pp60^{c-src} specific tyrosylkinase activity using a monoclonal antibody that recognizes the rat c-src protein¹⁰ (Fig. 2). ³²P-incorporation into pp60^{c-src} was used as a measure of the relative levels of pp60^{c-src} kinase activity. Neuronal lysates exhibited 8- to 10-times higher levels of ³²P incorporation into pp60^{c-src} than lysates from the cultured astrocytes, 4- to 6-times higher levels than lysates from embryonic brain tissue, and at least 20- to 30-times higher levels than lysates from embryonic limb tissue. Similar differences in the levels of kinase activity were detected when enolase was included in the assays to measure exogenous substrate phosphorylation¹¹. High levels of c-src protein kinase activity were maintained in the neuronal cultures that were carried in culture for as long as 4 months¹¹. These results indicate that the post-mitotic CNS neurones express and maintain high levels of the c-src protein kinase activity.

The higher levels of pp60^{c-src}-specific enolase phosphorylation detected in lysates from neurones compared with astrocytes could reflect differences in the steady-state levels of pp60^{c-src}, or differences in the specific activity of pp60^{c-src}. To examine these possibilities, we quantified levels of pp60^{c-src} protein and pp60^{c-src} kinase activity in neuronal and astrocyte cultures labelled with ³⁵S-methionine for 48 h (Fig. 3). Both the neuronal and astrocytic cultures contained 15- to 20-times higher levels of ³⁵S-methionine labelled pp60^{c-src} than rat fibroblast cultures, and contained levels comparable with those detected in RSV-transformed mouse cells (RSV-3T3 cells) (Fig. 3B). Since the ratio of trichloroacetic acid (TCA)-precipitable ³⁵S-c.p.m. per µg protein was similar for all of the cell lysates, the difference in the levels of ³⁵S-labelled pp60^{c-src} reflect the actual differences in the steady-state levels of pp60^{c-src}. Figure 3A shows that the pp60^{c-src}-specific phosphorylation of enolase was 6-times higher in lysates from neurones than in those from astrocytes. In replica experiments, lysates from neuronal cultures were found to possess 6- to 12-times higher levels of activity than lysates from astrocytes and 3-times lower than lysates from RSV-transformed cells. Since the levels of ³⁵S-methionine labelled pp60^{c-src} detected in neurones, astrocytes and RSV-transformed cells are similar, these results indicate that the specific activity of pp60^{c-src} from neurones is 6- to 12-times higher than that of astrocytes and 3-times lower than that of pp60^{v-src} in RSV-transformed mouse cells.

Lysates from neural retina and brain tissues contain a structurally distinct form of the cellular src protein that migrates as an electrophoretic variant of pp60^{c-src} on SDS-polyacrylamide gels¹². The alteration responsible for the shift in electrophoretic mobility maps within the aminoterminal region of pp60^{c-src}. To determine the cell-type producing this electrophoretic variant, the pp60^{c-src} protein was immunoprecipitated from lysates of ³²P-labelled neuronal, astrocytic or fibroblastic cultures (Fig. 4A). The proteins of molecular weight 60,000 were then subjected to partial proteolytic peptide mapping using *Staphylococcus* V8 protease (Fig. 4B). Figure 4C shows the pattern of cleavage of ³²P-labelled pp60^{c-src}. We have previously found that the V3 and V4 peptides derived from the pp60 protein from neural tissues migrate slightly more slowly than the analogous peptides of pp60 from CEFs or non-neural tissues, such as limb, liver, and heart¹². The V8 protease cleavage profile for the src protein immunoprecipitated from astrocytes is identical to that of pp60^{c-src} from fibroblasts. In contrast, the aminoterminal V3 and V4 peptides from the pp60^{c-src} protein expressed in neurons migrated more slowly than the V3 and V4 from the fibroblast and astrocyte pp60^{c-src} proteins. This electrophoretic variance was also observed in lysates from embryonic chicken neural tissues after 5 days' incubation and in embryonic rat neural tissues¹¹. A small amount of ³²P-incorporation was detected in V3 and V4 peptides that comigrated with the fibroblast-specific V3 and V4 peptides. It is not clear whether these peptides are

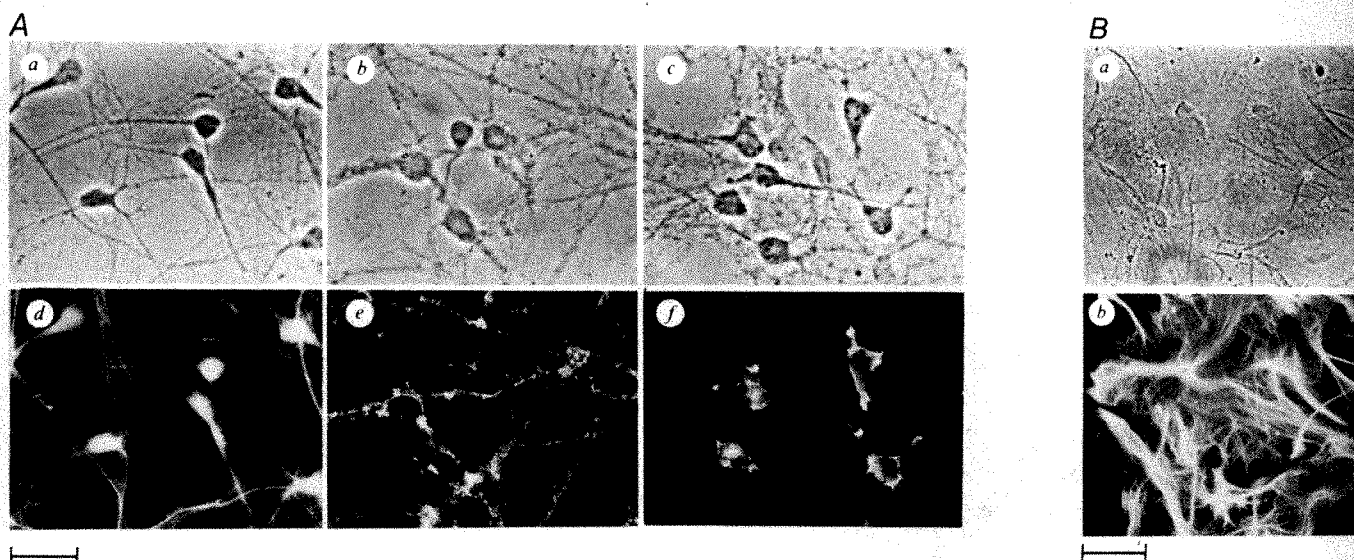
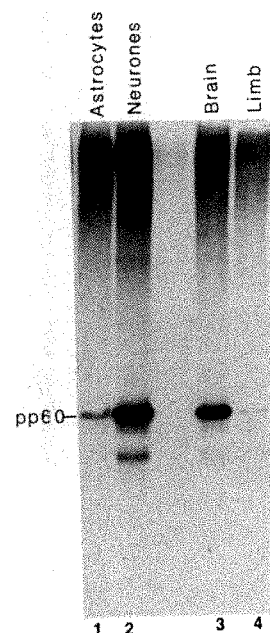


Fig. 1 Photomicrographs of cultures of neurones (A) and astrocytes (B) from embryonic and neonatal rat brain. A, For neurones, panels a-c show the phase contrast micrographs of the cells in d-f, which were stained with antibodies against neurofilament (NF)⁷ (d), tetanus toxin⁶ (e), or A2B5 antigen⁹ (f). For astrocytes (B) panel a shows the phase contrast micrograph of the cells in panel b stained with antibodies to glial fibrillary acidic protein (GFAP)⁸. Scale bar 20 μ m.

Methods. A, Neuronal cultures were obtained by dissociation of the metencephalon and lower telencephalon from 14-day Sprague-Dawley rat embryos. The tissue was disrupted into a cell suspension by gentle trituration and the cells were grown on collagen-polylysine coated 60-mm tissue culture dishes in N5 medium supplemented with 5% neurotropic fraction of horse serum as described⁵. For neurofilament staining (d), the cells were first fixed for 5 min in 10% buffered formalin and permeabilized with 95% ethanol. Cells were visualized using rabbit antiserum with 95% ethanol. Cells were visualized using rabbit antiserum to human NF protein, followed by fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit immunoglobulins (Ig). Tetanus toxin positive cells were visualized by incubation with tetanus toxin (2.5 μ g ml⁻¹) followed by horse anti-tetanus toxin and FITC-conjugated goat anti-horse whole serum. A2B5⁺ cells were visualized with monoclonal antibodies to A2B5 followed by tetramethyl-rhodamine isothiocyanate (TRITC) conjugated goat anti-mouse Ig. B, Astrocyte cultures were obtained by dissociation of the cerebral cortices from 1-day old Sprague-Dawley rats. The tissue was freed of meninges, and partially dissociated with pancreatin (1 mg ml⁻¹ in Ca²⁺/Mg²⁺-free Tyrodes) for 10 min. The cell suspension was centrifuged, and the pellet was dissociated by gentle repetitive pipetting and plated on collagen-polylysine coated 60-mm tissue culture dishes. Cultures were grown to confluency in N5 medium supplemented with 5% horse serum. Confluent cultures were treated with 10⁻⁵ M cytosine arabinose for 48 h to kill rapidly dividing cells. Cells were fixed and permeabilized as above and stained with rabbit antiserum against GFAP followed by FITC-conjugated goat anti-rabbit Ig. The percentage of cells binding antibodies was determined by counting approximately 1,000 cells per dish in four separate areas of the culture.

Fig. 2 Analysis of pp60^{c-src}-specific kinase activity of lysates of cultured neurones and astrocytes. Lane 1, astrocytes; 2, neurones; 3, adult mouse brain; 4, adult mouse limb. No 60K phosphoproteins were detectable in control immunoprecipitates obtained using anti-mouse Ig alone (see Fig. 3B, lanes 2, 4 and 7).

Methods. Cultures of neurones and of astrocytes were prepared as in Fig. 1 and lysed in 1 ml SLB detergent lysis buffer (1% sodium deoxycholate, 1% Nonidet, 0.075 mM NaCl, 5 mM EDTA, 10 mM Tris-HCl pH 7.0, 1 mM ethylene glycol-bis[β -aminoethyl ether]-N,N-tetraacetic acid) and clarified at 49,000g for 30 min. The protein concentration was then determined by the method of Lowry *et al.*³⁰ and samples were normalized for protein concentration (200 μ g) by the addition of SLB lysis buffer. pp60^{c-src} was then immunoprecipitated from the lysates and assayed for autophosphorylation according to Lipsich *et al.*¹⁰ using 1 μ l of a 1:100 dilution of ascites fluid from hybridoma 327 (MAb327) and 1 μ g of rabbit anti-mouse Ig. The kinase reaction was performed for 15 min at 22°C in kinase assay buffer (5 μ C [γ -³²P]ATP, 5 uM ATP, 10 mM Tris-HCl pH 7.4, and 5 mM MgCl₂). Samples were electrophoresed on a 7.5% Laemmli SDS-polyacrylamide gel³¹ and were autoradiographed with XAR (Kodak) film and a Lightning-Plus screen for 15 h.



derived from nonmodified *src* protein molecules present in the cultured neurones or from pp60 expressed in non-neuronal cells present in the neuronal cultures. This variant form of pp60^{c-src} was detected using three different antisera that recognize pp60^{c-src} (data not shown).

These results indicate that cultured neurones and astrocytes contain high levels of the pp60^{c-src} protein. However, the *c-src* protein expressed in neuronal cultures can be distinguished from the protein expressed in astrocytes in two ways: (1) the pp60^{c-src}

protein from neurones exhibits a 6- to 12-times higher specific activity of tyrosine phosphorylation than the protein from astrocytes, and (2) the pp60^{c-src} protein from neurones contains a structural alteration within the amino-terminal domain of the molecule. These results provide the first example of cell-type specific differences in the functional activity of a cellular proto-oncogene that involves structural modification of the protein rather than increased levels of the protein.

A correlation in activation of the *c-src* has also been observed

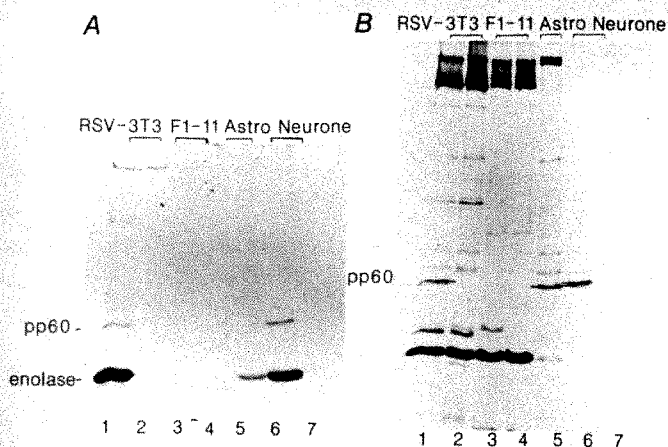


Fig. 3 Comparison of the specific activity of the cellular *src* protein in neurones and astrocytes. Electrophoresis sample buffer was added to half of the immunoprecipitate for analysis of the ^{35}S -methionine labelled $\text{pp60}^{\text{c-src}}$ (panel B), and the other half was assayed for enolase phosphorylation (panel A) by the addition of 2 μg of enolase and kinase reaction mix as described in Fig. 2. RSV-transformed mouse cells (lanes 1, 2); rat fibroblasts (F1-11 cells) (lanes 3, 4); rat astrocytes (lane 5); rat neurones (lanes 6, 7). **Methods.** Cultures of neurones and astrocytes were prepared as described in Fig. 1. These cells and rat F1-11 fibroblasts (from P. Jat, Massachusetts Institute of Technology) and RSV-transformed mouse 3T3 cells were labelled for 48 h with 100 μCi of ^{35}S -methionine in methionine-free medium supplemented with complete Dulbecco's minimum essential medium to 10% of the total volume. The medium was replenished after 24 h. The cells were lysed in SLB lysis buffer and the lysate clarified as described elsewhere³². The incorporation of ^{35}S -methionine into protein was assayed by TCA precipitation and the protein concentration was determined using the Lowry assay³⁰. The ratio of TCA-precipitable c.p.m. of ^{35}S -methionine to total protein was similar in all cell lysates. Sixty micrograms of each lysate was incubated with 1 μl MAb 327 and 1 μg of anti-mouse Ig (lanes 1, 3, 5, 6) or anti-mouse Ig alone as control (lanes 2, 4, 7). After washing, the immunoprecipitates were split. The levels of enolase phosphorylation were quantified by determining the c.p.m. of ^{32}P in excised gel pieces. (RSV-transformed cells, 7,270 c.p.m.; neurones, 2,480 c.p.m.; astrocytes, 402 c.p.m.; fibroblasts, 70 b.p.m.). The relative incorporation of ^{35}S -methionine into $\text{pp60}^{\text{c-src}}$ was determined by densitometric scanning of the 60K region of the autoradiogram using a Joyce-Loebbel Ephortec densitometer in the Rascor scan mode. In this experiment, the levels of incorporation of ^{35}S -methionine were equivalent for $\text{pp60}^{\text{c-src}}$ from the astrocyte and RSV-transformed cell cultures, 1.4-times higher in the neurone cultures, and 19-times lower in the fibroblast cultures.

between polyoma virus transformed cells and mouse 3T3 cells treated with platelet-derived-growth-factor (PDGF). The *c-src* protein associated with the middle T antigen of polyoma virus possesses 30- to 50-times higher levels of kinase activity and migrates with an electrophoretic mobility similar to that of $\text{pp60}^{\text{c-src}}$ from neurones^{13,14}. Ralston and Bishop have found that the *c-src* protein from mouse 3T3 cells treated with PDGF also contains an amino-terminal modification that alters both the electrophoretic mobility and the tyrosine kinase activity of $\text{pp60}^{\text{c-src}}$ (R. Ralston and J. M. Bishop, personal communication). In both of the latter systems, the modified aminoterminal fragment of $\text{pp60}^{\text{c-src}}$ was found to contain phosphotyrosine, which is not detectable in the unmodified form of the *c-src* protein. In contrast, we have not detected phosphotyrosine in the amino-terminal V8-protease fragments of $\text{pp60}^{\text{c-src}}$ expressed in neurones¹². It is possible that the activity of $\text{pp60}^{\text{c-src}}$ can be influenced by either tyrosine phosphorylation or other modifications within the amino-terminal domain of the molecule. Iba and Hanafusa have found that the *c-src* gene expressed by nontransforming retroviruses mutates at a high frequency¹⁵ to generate variants possessing elevated kinase activity¹⁶, which are capable of efficient transformation of fibroblasts¹⁵, suggest-

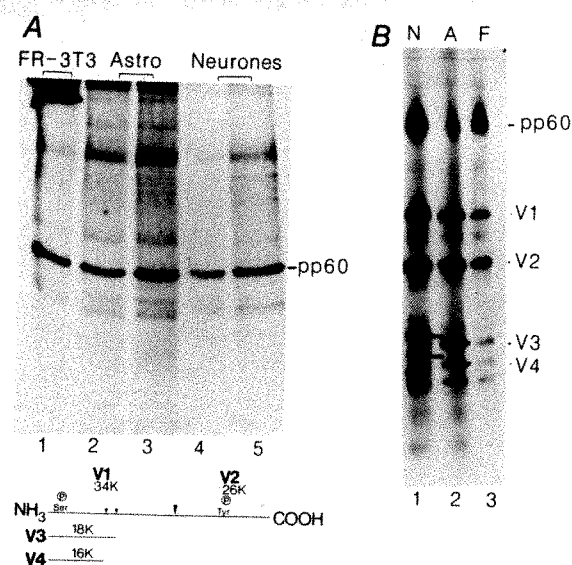


Fig. 4 Limited proteolysis of $\text{pp60}^{\text{c-src}}$ immunoprecipitated from lysates of cultured neurones and astrocytes. **A**, Cultures of neurones and astrocytes were prepared as described in Fig. 1. These cultures and Fisher rat 3T3 cells were labelled with ^{32}P (1 mCi ml^{-1}) for 4 h. Samples were lysed and $\text{pp60}^{\text{c-src}}$ was immunoprecipitated as described in Fig. 2 using 1 μl MAb 327 (1:100). The immunoprecipitates were electrophoresed on a 7.5% Laemmli SDS-polyacrylamide gel³¹. Lane 1, Fisher rat 3T3 cells; lanes 2 and 3, astrocytes; lanes 4 and 5, neurones. A doublet of $\text{pp60}^{\text{c-src}}$ is observed in lanes 4 and 5. **B**, Partial proteolysis of $\text{pp60}^{\text{c-src}}$. $\text{pp60}^{\text{c-src}}$ was excised and mapped by partial peptide proteolysis with 50 ng *Staphylococcus aureus* V-8 protease on a 12.5% SDS-polyacrylamide gel using the method of Cleveland³³. Following electrophoresis, samples were autoradiographed with XAR (Kodak) film and a Lightning-Plus screen. Lane 1, neurones; lane 2, astrocytes; lane 3, NIH Fisher rat 3T3 cells. The lines designate V-3' and V-4' peptides in lane 1. The dots indicate the V-3 and V-4 peptides in lanes 2 and 3. **C**, A map of $\text{pp60}^{\text{c-src}}$ showing the cleavage pattern observed following treatment with *Staphylococcus aureus* V-8 protease. Partial proteolytic digestion using low concentrations of V-8 protease cleaves the pp60 molecule into two fragments³⁴: (1) a peptide of M_r 34,000 (designated V1) which is phosphorylated on serine and contains the amino terminus of pp60 , and (2) a peptide of M_r 26,000 (designated V2) that is phosphorylated on tyrosine³⁵ and contains the carboxylterminus of $\text{pp60}^{\text{c-src}}$. Peptides of M_r 18,000 and 16,000 (designated V3 and V4) are generated by further cleavage of the V-1 peptide and both contain the aminoterminal of pp60 .

ing a large target region within $\text{pp60}^{\text{c-src}}$ in which mutations can activate the tyrosine kinase activity of $\text{pp60}^{\text{c-src}}$.

The expression of high levels of *c-src* kinase activity in post-mitotic neurones indicates that activation of *c-src* expression does not correlate with cell proliferation. This observation raises the question of whether either the *c-src* protein serves a specific function in neurones or the *c-src* protein is involved in the initiation or maintenance of neuronal differentiation. This result contrasts the functional activity of the viral *src* protein, which interferes with differentiation of neuroectoderm cells¹⁷, and other types of immature precursor cells¹⁸⁻²¹. The basis for this discrepancy is not known; however, recent evidence indicates that the viral *src* protein is functionally distinct from the cellular *src* protein. Although both proteins possess tyrosine specific kinase activity²²⁻²⁷, several laboratories have found that overexpression of the *c-src* protein does not mimic the transforming effects of the viral *src* protein^{15,28,29} and the phosphotransferase activity of the *c-src* protein is qualitatively and quantitatively different from that of the viral *src* protein (ref. 16; P. M. Coussens and D. Shalloway, personal communication). These differences in kinase activity could lead to changes in the substrate specificity of the *v-src* protein compared with the cellular

src protein *in vivo*, thus eliciting cellular responses not brought about by *c-src*-mediated tyrosine phosphorylation of cellular proteins.

Identification of neurones as the type of cell that specifically expresses high levels of the *c-src* protein kinase activity provides a focus for future investigation of the function of the cellular *src* protein. Neurones could provide an ideal system to probe the function of *c-src* since the physiology of these cells is so well characterized. In addition, the availability of cell cultures of neurones containing minimal contamination from other neural or stromal cell types offers many advantages over the use of whole tissues.

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Differentiation of PC12 phaeochromocytoma cells induced by *v-src* oncogene

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PC12 rat phaeochromocytoma cells^{1,2} are a model system that can be used to study both neuronal differentiation and the mechanism of action of nerve growth factor (NGF)³. PC12 cells respond to NGF protein by shifting from a chromaffin-cell-like phenotype to a neurite-bearing sympathetic neurone-like phenotype^{2,4,5}. Here we present data on the effect of infection of PC12 cells with retroviruses carrying the *src* oncogene of Rous sarcoma virus⁶. Previous studies have demonstrated that the expression of *src* severely affects the synthesis and accumulation of differentiated cell products in a variety of cell types⁷⁻⁹. We show that in the PC12 cell system, expression of *v-src* appears to have an inductive effect on differentiation that resembles the action of a 'physiological' growth factor.

PC12 cells grown without exogenously supplied NGF do not possess neurites. When NGF is added to cultures, there is a lag of about 24 h before neurites are first detectable, after which they appear over several days; by this time >95% of cells possess neurites¹. PC12 cells, cultured on collagen-coated dishes as described previously¹, were infected with the mammaltropic strain Schmidt-Ruppin D (SR-D) of Rous sarcoma virus (RSV) (multiplicity of infection (MOI) between 1 and 10 as assayed on susceptible chicken embryo fibroblasts). After a lag of about 48-72 h after infection, a fraction of the cells started to undergo a marked phenotypic conversion, shown by the emission of short and long neurites, as in NGF-treated cells (Fig. 1). Eventually, after 7-10 days, a variable proportion of infected cells (up to 30%, depending on MOI and experiment) exhibited long neurites. Differentiated cells conserved their phenotype in culture for several days, and could be replated after trypsinization. Severed neurites re-grew within 24-48 h. Parallel experiments were performed using PC12 cells that had been pretreated with

mitomycin C (1 µg ml⁻¹ for 18 h), thus eliminating further cell proliferation. In mitomycin C-treated cells, only neurite extension promoted by NGF took place, but its time course was considerably shortened, thus reinforcing the notion that division is not required for differentiation in this system⁴. On the contrary, infection with SR-D failed to elicit a differentiated phenotype in mitomycin C-treated PC12 cells, suggesting that establishment of infection¹⁰ is a necessary event (data not shown). Antibodies directed against NGF, although capable of fully inhibiting NGF-induced neurite extension, did not inhibit the outgrowth elicited by RSV infection. Conversely, putatively infected cells that did not emit short or long neurites continued to divide actively and were competent to extend fibres after cultivation in the presence of NGF. When tissue culture fluids from SR-D-infected PC12 cultures were tested for induction of morphological differentiation in uninfected cells, no neurite extension was detected in such cultures, making it unlikely that the observed phenomenon was a result of the release of long-range acting substances endowed with NGF-like properties.

Further evidence that differentiation of RSV-infected cells is under the continuous control of the *src* gene was provided by a genetic analysis using a variety of mammaltropic viral strains and their conditional mutants⁶. Thus, both the B77 strain of RSV and a murine recombinant retrovirus containing the *src* gene (MRSV)¹¹ were capable of inducing morphological differentiation of PC12 cells with similar kinetics to those exhibited by SR-D, albeit with a lower efficiency (Fig. 1d). PC12 cells, infected with the temperature-sensitive (*ts*) mutant LA339-B77 (ref. 12) and passaged at 39.5 °C, were indistinguishable from uninfected controls. Following a shift to 35 °C, however, the *ts*-LA339-infected cells within 2-3 days underwent a morphological transition to neurone-like cells with long processes (not shown). Infection with transformation-defective (*td*) B77 alone as well as with the non-mammaltropic strains SR-A and *ts*-LA24 did not produce any alteration in the morphology or growth behaviour of the cells. To substantiate that the emission of neurites was under the control of the *src* gene, we used the immune complex kinase assay¹³ to test for the presence of the *src* primary product, pp60^{v-src} (ref. 14), in RSV-infected cultures (Fig. 2). SR-D-infected PC12 cells at 5 days post-infection contained elevated levels of pp60^{v-src} (Fig. 2a), comparable with those of a SR-D-transformed clone of the rat myogenic cell line

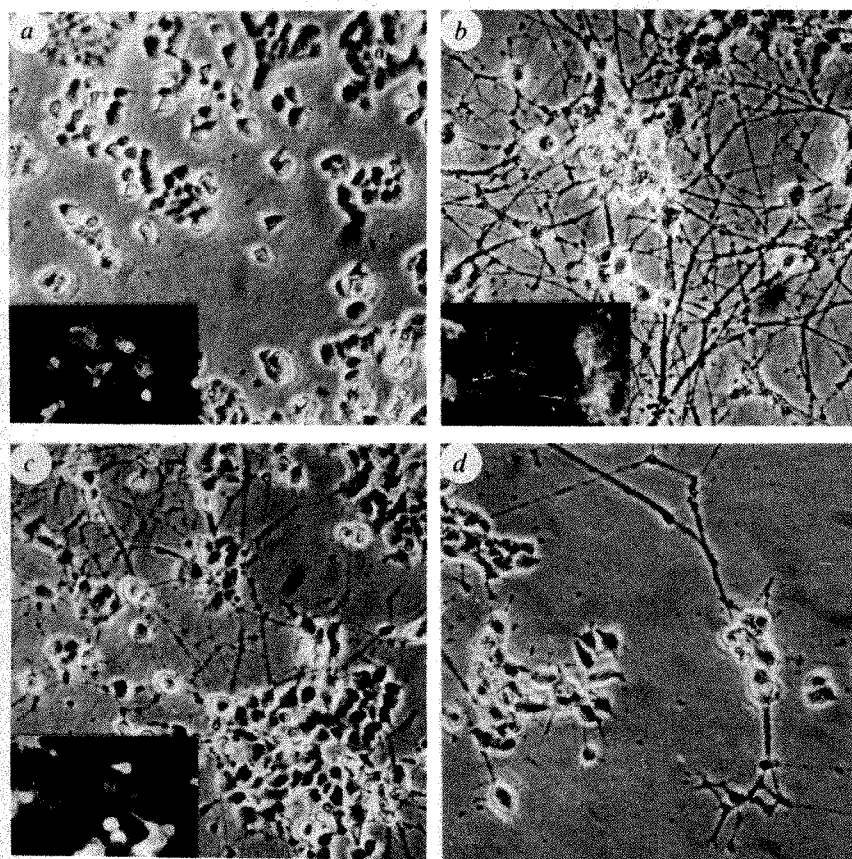


Fig. 1 Phase-contrast and catecholamine fluorescence (insets) micrographs of fixed PC12 cells undergoing differentiation. *a*, Control, uninfected PC12 cells; *b*, NGF-treated cells; *c*, SR-D-infected cells; *d*, MRSV-infected cells, enriched through serum deprivation. The percentage of differentiated cells with long (beyond two cell body diameters) neurites was difficult to assess because PC12 cells have a strong tendency to aggregate. It was often possible to see many neurites emerging from cellular aggregates composed of tens of cells. Neurites in RSV-infected cultures possessed flattened growth cone-like structures and often had varicosities, which are a specific attribute of sympathetic-like cells, thus distinguishing RSV-induced processes from the short ephemeral extensions produced following certain treatments³¹.

Methods. PC12 cells were infected with RSV at a MOI of 1–10 in the presence of 2 μ g polybrene per ml and thereafter grown in standard culture conditions or in the absence of serum. After attaining confluence, cultures were trypsinized and seeded in triplicate at 2×10^5 cells per 35-mm collagen-coated dishes and further incubated at the appropriate temperature for 5 days. NGF-treated cells were cultured in medium supplemented with 100 ng per ml of 2.5S NGF³² (a kind gift of D. Mercanti); the medium was changed every third day. Photographs were taken after fixation with 3% paraformaldehyde. Catecholamines were visualized by the glyoxylic acid method³³.

L8 (ref. 15). In contrast, pp60^{v-src} was expressed at significantly lower levels in cultures of Rat-1 cells freshly (5 days) infected with SR-D at the same MOI (Fig. 2*a*). Levels of pp60^{c-src}, the cellular *src* kinase activity¹⁶, remained constant when assayed 10 days after addition of NGF or infection by RSV (Fig. 2*b*).

SR-D-infected and differentiated cells remain viable when cultivated in serum-free conditions, whereas undifferentiated ones die within a few days. This property, also shared by NGF-treated PC12 cells¹⁷, has been exploited as a simple means to obtain cultures highly enriched in differentiated cells, and suitable for assaying the expression of two constitutive markers of the PC12 cell line: storage of catecholamines and α -bungarotoxin binding sites^{2,18}. Neither marker was significantly altered (Fig. 1, insets and data not shown), suggesting that in these cells expression of high levels of pp60^{v-src} does not interfere with the differentiative programme.

We next investigated whether morphologically differentiated cells can synthesize DNA or whether differentiation is accompanied by a progressive withdrawal from the cell cycle, as shown previously for NGF-treated PC12 cells². This was monitored by pulsing the cells with ³H-thymidine in high serum for 48 h and locating labelled cells by autoradiography. The autoradiographic studies showed that while most control PC12 cells, grown in serum, were labelled, a considerable proportion of serum-free enriched, differentiated cells, bearing neurites many cell body diameters in length, had ceased to incorporate ³H-thymidine (Fig. 3). Differentiated PC12 cells, selected through serum deprivation, showed a further three-fold increase in pp60^{v-src} specific activity, a finding which indicates strongly that differentiated cells do express levels of kinase usually associated with transformation in other rat cell types (see SR-D-L8 in Fig. 2).

Our results show that while expression of v-*src* in the PC12 cells system can efficiently induce these cells to progress further along their differentiative pathway, it does not seem to suppress two specific differentiation markers constitutively expressed in the cell line. This may seem surprising in view of the well-known effects of *src* in a wide range of RSV-transformed avian cell types in culture. In all systems in which *src* has a demonstrated

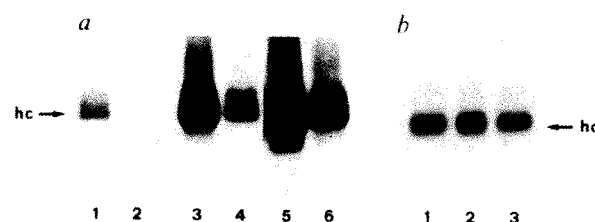
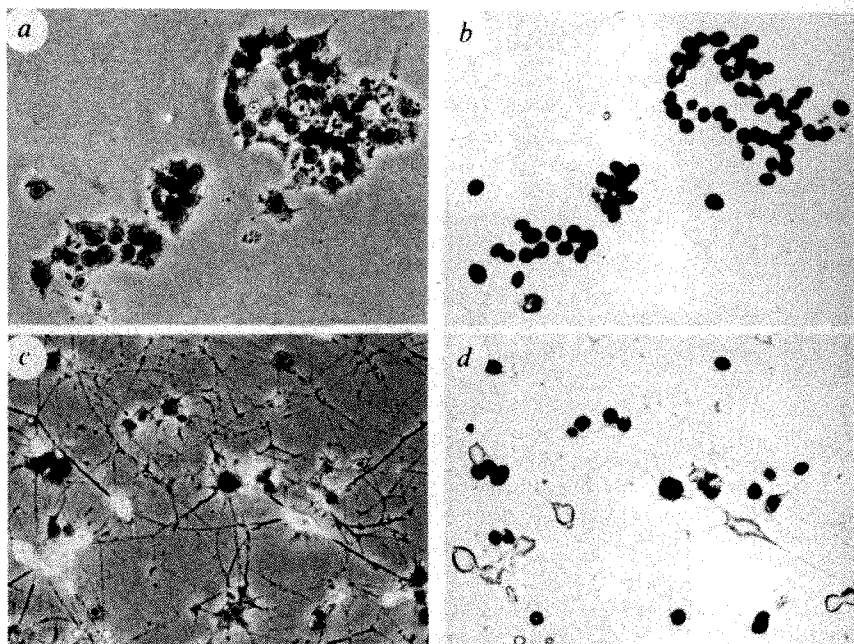


Fig. 2 Expression of pp60^{src} in uninfected and RSV-infected PC12, Rat-1 and L8 cells. *a*, pp60^{v-src} kinase activity in cell extracts of SR-D-infected Rat-1 (lanes 1, 2) and PC12 cells (lanes 3, 4) 5 days post-infection; SR-D-transformed L8, clone F (lanes 5, 6). Lanes 2, 4 and 6 are assays carried out on extracts diluted 1 to 5 in order to check linearity of assay. *b*, pp60^{c-src} activity in extracts of control uninfected (lane 1), NGF-treated (lane 2) and SR-D-infected (lane 3) PC12 cells. hc, IgG heavy chain.

Methods. PC12 and Rat-1 cells were cultured and infected as described in Fig. 1 legend. L8 cells, a rat myogenic cell line, were infected by SR-D and transformed cells were isolated and cloned as described in ref. 15. The *src*-encoded transforming protein has been demonstrated to possess kinase activity capable of phosphorylating the heavy chain of IgG. The *src* kinase activity was measured in cultures washed with phosphate-buffered saline and frozen *in situ* at -80°C . Briefly, cells were lysed in 1% NP40-containing buffer, also containing 0.2 mM phenylmethylsulphonyl fluoride. After clarification at 80,000g for 30 min, aliquots of extracts, normalized for total protein content (200–500 $\mu\text{g ml}^{-1}$), were challenged with 5 $\mu\text{l ml}^{-1}$ of two different tumour-bearing rabbit sera. The first antiserum (obtained from P. Enrietto) recognizes pp60^{src} of Prague-A strains of RSV and cellular *src* but does not cross-react significantly with pp60^{src} from the SR-D strain. The second antiserum (obtained from B. Friis) reacts poorly with pp60^{v-src} but precipitates pp60^{v-src} from both SR-D and Prague-A strains. The combined use of the two antisera allows the detection of relative variations in the kinase activity of the viral (*a*) and cellular (*b*) kinases without mutual interference. Immune complexes were reacted at 20°C for 10 min in 50 mM HEPES buffer, pH 7.5, 5 mM MgCl₂, 5 mM MnCl₂, 4 μCi of [γ -³²P]ATP and then analysed on 10% SDS-polyacrylamide gels.

Fig. 3 Micrographs of representative fields of autoradiographs from cultures of control (a, b) or SR-D-infected (c, d) PC12 cells. PC12 cells that had been cultured for 48 h either in serum-free medium (SR-D-infected) or in 10% fetal calf serum (control cultures) were transferred to high serum and incubated for 48 h with ^3H -thymidine ($2\ \mu\text{Ci ml}^{-1}$, $40\ \text{Ci mmol}^{-1}$, NEN). The cells were then fixed and processed for autoradiography as described elsewhere⁹. Phase-contrast (a, c) and bright-field (b, d) pictures of the same field are shown to better contrast ^3H -thymidine incorporation in nuclei and morphology of differentiated cells.



effect on differentiation, the effect seems to involve a direct and specific suppression of a given differentiation programme^{7-9,19,20}. It was recently shown, however, that macrophages could be infected by RSV, without discernible alterations in proliferative or differentiative parameters²¹. Neither the failure of macrophages to transform nor the ability of PC12 cells to differentiate can be explained by reduced levels of pp60^{v-src} (ref. 22 and Fig. 2). These cells may, however, lack a developmentally regulated (lineage-specific) function, which is essential in the pathway leading other cell types to transformation²².

The vertebrate *c-src* gene is expressed at high levels and in a stage- and cell-type-specific fashion in the nervous system^{23,24}, a finding that has led to the hypothesis that pp60^{c-src} may be involved in the control of the differentiative processes of neural crest-derived cells²⁴. Whether this speculative argument is compatible with the available evidence remains to be assessed. For the moment, the present results provide some qualitative supporting evidence, notwithstanding the known structural and functional differences between *c-* and *v-src* gene products²⁵. In this context, it is noteworthy that expression of the *c-fos* proto-oncogene, while causing transformation in mammalian fibroblasts²⁶, promotes differentiation of teratocarcinoma stem cells²⁷.

A final intriguing question concerns the possible relationship between the mechanism of action of NGF and the biochemical events that accompany the activity of pp60^{v-src}, such as tyrosine phosphorylation of proteins or increased turnover of the phosphoinositides²⁹. Experiments are in progress to test this hypothesis and whether *v-src* triggers an aberrant type of differentiation in PC12 cells or shares a common pathway with NGF. However, receptor-bound growth factors such as epidermal growth factor share similar transduction events with *v-onc*-encoded transforming proteins²⁹, yet do not induce differentiation of PC12 cells³⁰. Therefore, there seems to be a high degree of molecular specificity in the multiple responses triggered by NGF and *v-src*.

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Induction of proliferation or transformation of neuroretina cells by the *mil* and *myc* viral oncogenes

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The genome of the avian retrovirus MH2 contains, in addition to the *v-myc* oncogene shared with three other avian retroviruses (MC29, CMII and OK-10), a second cell-derived oncogene, *v-mil* (refs 1-3). Like the three other viruses, which contain only *v-myc*, MH2 induces mainly liver and kidney carcinomas in fowl and

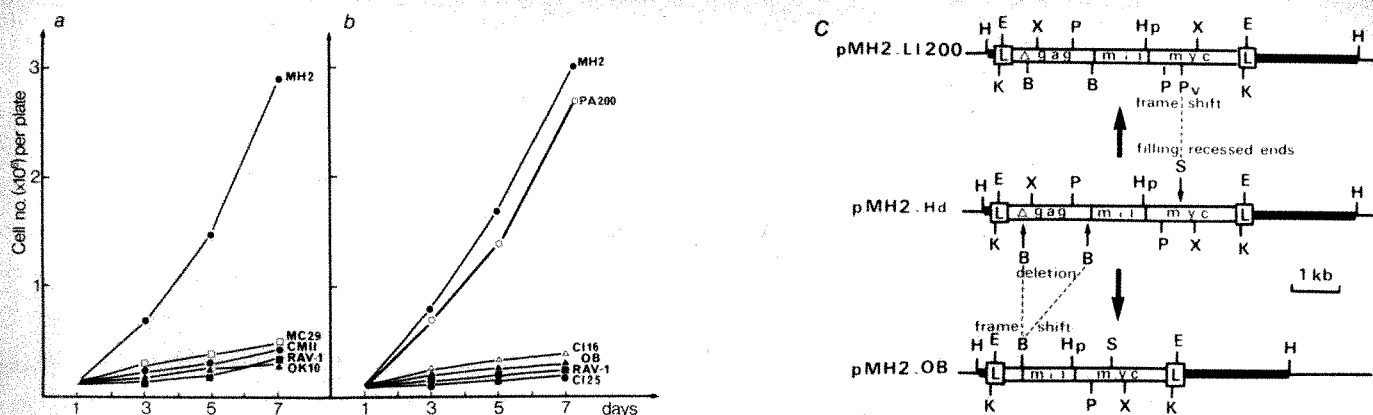


Fig. 1 Growth kinetics of virus-infected chicken neuroretina cells. NR cells were infected with MH2 (wild-type), MC29, CMII, OK-10 and RAV-1 (a) and MH2, MH2-PA200, MH2-cl.16, MH2-cl.25, MH2-OB and RAV-1 (b), then seeded at low density ($\sim 2 \times 10^5$ cells per 5-cm dish). Medium was renewed daily and cells from two aliquot dishes were counted as indicated. Viruses were obtained by RAV-1 superinfection of non-producer clones. (c) Constructed mutants. B, *Bam*HI; E, *Eco*RI; H, *Hind*III; Hp, *Hpa*I; K, *Kpn*I; P, *Pst*I; Pv, *Pvu*I; X, *Xho*I; L, long terminal repeat. Open boxes represent proviral sequences and closed boxes flanking cellular sequences.

Methods. Preparation of NR cell cultures and growth assay: Neuroretinas were dissected from 7-day-old chick embryos. Cells were dissociated and resuspended in Eagle's basal medium (BME) supplemented with 5% fetal calf serum. Dishes (35 mm) containing $\sim 2 \times 10^6$ cells were infected with the various viruses. When proliferation became evident in wild-type MH2-infected cells (7–10 days post-infection), cultures were passaged once in 5-cm dishes until MH2-infected cells reached confluency, and then seeded at low density for growth assay. Isolation of class I mutants MH2-cl.16, MH2-cl.25: Two viral stocks were used. MH2 (RAV-1) was obtained by superinfecting a non-producer quail embryo fibroblast (QEF cells) clone, MH2-QB2 (ref. 11), with RAV-1. MH2 (RAV-3) was kindly provided by Dr C. Moscovici. Both viruses exhibited comparable mitogenic and transforming properties in NR cells and directed the synthesis of P100^{gag-mil} in transformed cells. QEF cells were infected at low multiplicity with MH2 (RAV-1) or MH2 (RAV-3) and seeded 6 h later at low density in soft agar-containing medium. Colonies of transformed cells developed within 2 weeks and were individually transferred to 35-mm dishes. At confluency, RAV-1 was added to rescue virus from occasional non-producer clones. Supernatants were used to infect NR cells. Media from two such clones, 16 and 25, contained virus which failed to induce NR cell growth. MH2-cl.16 originated from MH2 (RAV-1) and MH2-cl.25 from MH2 (RAV-3). The two mutant viruses were subcloned on QEF cells in soft agar, and virus stocks rescued from non-producer colonies by RAV-1 superinfection were unable to induce NR cell multiplication, but transformed chicken fibroblasts and macrophages. Isolation of class II mutant MH2-PA200: MH2-PA200 was isolated by end-point dilution of MH2 on NR cells. NR cells were infected with serial twofold dilutions of MH2 (RAV-1), to determine the highest dilution of the virus inducing cell proliferation. The reciprocal of this dilution defined the mitogenic titre of the virus in mitogenic units (MU) per ml. Several NR cultures were then infected with 0.3–0.5 MU per plate and superinfected 1 week later with RAV-1 to rescue virus from occasional non-producer cells. Virus produced by one such culture was selected for this study because it induced NR cell multiplication in the absence of morphological transformation. This variant was designated MH2-PA200 (RAV-1). Strategy used for constructed mutants (c): DNA of pMH2-Hd plasmid¹ was digested with *Bam*HI, ligated and transfected in *Escherichia coli* HB101 cells. A clone was selected, pMH2-OB, which lacked the 1.3-kb *Bam*HI restriction fragment derived from the *gag* gene. Sequence analysis around the recombination site indicated that translation of the pMH2-OB RNA should result in the synthesis of a product containing the amino-terminal 52 amino acids of p19^{gag} linked to 5 amino acids derived from the *v-mil* sequence in a +1 frameshift (C. Henry and B. Debuire, unpublished data). To construct the MH2-LI200 mutant, DNA of pMH2-Hd plasmid was partially cleaved by *Sal*I endonuclease and cohesive termini were filled in by treatment with the Klenow fragment of DNA polymerase I. This generated a new *Pvu*I site as expected, and should result in a +1 frameshift in the translation of the *v-myc* sequence. Infectious virus (MH2-OB) was rescued from pMH2-OB DNA transfected cells using RAV-1 as helper virus.

transforms fibroblasts and macrophages *in vitro*⁴. However, MH2 and MC29 differ in their biological properties when assayed on cultures of chicken embryo neuroretina (NR) cells. Indeed, NR cells, which normally do not multiply *in vitro*, are induced to proliferate and become transformed upon infection with MH2, whereas infection with MC29 has no apparent effect on these cells^{5,6}. To analyse the functions of the two oncogenes of MH2, we isolated spontaneous and *in vitro*-constructed mutants of this virus and investigated their effects on NR cell multiplication and transformation. We report here that expression of *v-mil* is sufficient to induce NR cell proliferation, although it does not result in cell transformation. In addition, viruses expressing only the *v-myc* oncogene fail to induce any detectable change in NR cells. However, cooperation of the two oncogenes is required to achieve transformation of NR cells by MH2.

Neuroretinas, dissected from 7-day-old chicken embryos, were dissociated into essentially single-cell suspensions and plated in Eagle's basal medium containing 5% fetal bovine serum. These cultures contain only neurones and glial cells^{7,8}. Infection of NR cells with MH2 resulted within 7–10 days in the appearance of actively proliferating cells which could be readily subcultured for several generations (Fig. 1a). These cells were morphologically transformed and displayed anchorage-independent growth capacity (results not shown). In contrast, NR cells infected with MC29, CMII or OK-10, or with the helper virus RAV-1 used as a control, retained the morphology and limited growth capacity of normal cells (Fig. 1a). This lack

of phenotypic response was not due to a block in virus replication, because the *v-myc* products were synthesized in infected cells and supernatants of infected NR cultures contained virus that efficiently transformed chicken embryo fibroblasts (results not shown).

To analyse the biological properties of the two oncogenes of MH2 further, we isolated two classes of virus mutants by their capacity to alter the phenotype of NR cells. Class I mutants were selected because they were unable to induce NR cell proliferation (see Fig. 1 legend). Two such mutants, MH2-cl.16 and MH2-cl.25, were isolated from two distinct viral stocks. MH2-cl.16 and MH2-cl.25, like wild-type MH2, transformed chicken embryo fibroblasts and macrophages (T. Graf, personal communication). In contrast to wild-type virus-infected cultures, NR cells infected with either mutant were not transformed, nor were they stimulated to grow (Fig. 1b). An additional mutant virus, MH2-OB, possessing similar biological properties (Fig. 1b), was obtained by deleting most of the *gag* sequence in plasmid pMH2-Hd (ref. 1) which contains a biologically active provirus (this yields a premature termination of the *gag-mil* protein, see Fig. 1c).

Two class II mutants were isolated as follows. Mutant virus MH2-PA200 was selected because it induced proliferation of NR cells, without morphological transformation (Fig. 1 legend). Such cells could be subcultured for many generations but ultimately did senesce (after ca. two months). The second mutant, MH2-LI200, with similar biological properties, was derived from

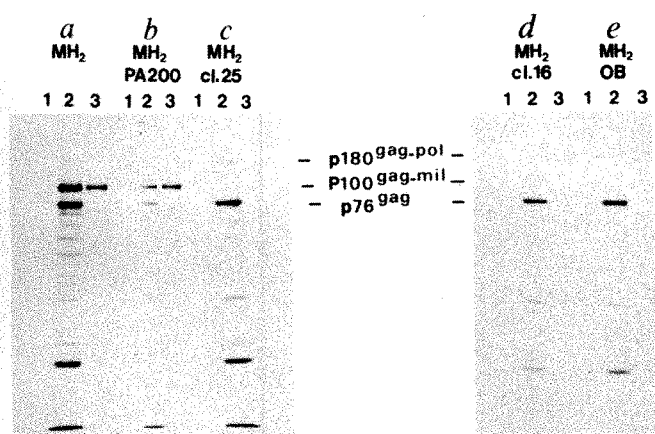


Fig. 2 Characterization of *gag* and *mil* related proteins synthesized in avian cells infected by wild-type and mutant MH2 viruses. Chicken NR cells transformed by wild-type MH2 (a), or infected by MH2-PA200 (b) and fibroblasts transformed by MH2-cl.25 (c), MH2-cl.16 (d) and MH2-OB (e) were incubated for 3 h in the presence of 30 $\mu\text{Ci ml}^{-1}$ of L-35S-methionine (specific activity 1,000 Ci mmol $^{-1}$), lysed and immunoprecipitates prepared as described previously²². Sera used were as follows: lanes 1, pre-immune rabbit serum; lanes 2, rabbit anti *gag* serum; lanes 3, rabbit antiserum prepared to a bacterially expressed protein corresponding to the carboxy terminus of P100^{gag-mil} (F.D. and J.G., in preparation). Immunoprecipitated proteins were analysed by SDS-polyacrylamide gel electrophoresis as described by Laemmli²³ followed by fluorography on the dried gel.

plasmid pMH2-Hd (ref. 1) by generating a frameshift mutation in *v-myc*, resulting in the premature termination of its translation product (Fig. 1c legend).

The P100^{gag-mil} translation product of *v-mil*^{9,10} was readily detected by immunoprecipitation of lysates of ³⁵S-methionine-labelled cells infected with either wild-type MH2 or class II mutants (that is, MH2-PA200, Fig. 2b) using antiserum specific for *gag* proteins (lanes 2) or for a bacterially expressed *v-mil* protein (lanes 3). In contrast, no *v-mil* specific protein was detected in fibroblasts transformed by class I mutants (Fig. 2c-e, respectively).

We next performed blot analyses of RNA isolated from cells infected with either wild-type or mutant viruses. As expected from previous studies^{1,11}, cells infected with wild-type MH2 synthesized a 5.7-kilobase (kb) genomic RNA detectable with either a *v-mil* or a *v-myc* probe (Fig. 3c, lanes 1 and 2, respectively) and a 2.8-kb subgenomic RNA detected with a *v-myc* probe only (Fig. 3c, lane 2). The 5.7-kb genomic RNA and the 2.8-kb subgenomic RNA species are known to encode respectively the P100^{gag-mil} and p60/61^{myc} proteins^{12,13}. The 2.8-kb *myc*-specific RNA was detected in fibroblasts transformed by MH2-cl.16, MH2-cl.25 and MH2-OB at levels similar to those observed in cells transformed by wild-type MH2 (Fig. 3a, b, d, lanes 2). No virus-specific RNA hybridizing to the *v-mil* probe was detected in cells infected with mutant virus MH2-cl.16 and MH2-cl.25, indicating that these mutants had lost most or all of the *v-mil* sequences. In addition to the 2.8-kb RNA, cells infected with MH2-OB synthesized a 4.4-kb RNA that hybridized to both *v-mil* and *v-myc* probes (Fig. 3d). This, together with the size of the fragment deleted to generate pMH2-OB (1.3-kb, see Fig. 1c), led us to conclude that the 4.4-kb RNA was the genomic RNA of MH2-OB. The 4.0-kb species detected by the *v-mil* probe corresponds to the previously described *c-mil* endogenous transcript in fibroblasts¹ and appears on long exposures (Fig. 3a, b, lanes 1).

In contrast, no RNA hybridizing to a *v-myc* probe was detected in NR cells infected with MH2-PA200 (Fig. 3e, lane 2). The only virus-specific RNA synthesized in these cells was a 5.7-kb RNA hybridizing to a *v-mil* probe (Fig. 3e, lane 1). This RNA also hybridized to an *env* probe (not shown). The MH2-PA200 provirus has been molecularly cloned and the restriction map of this provirus suggests that it has been generated by recombina-

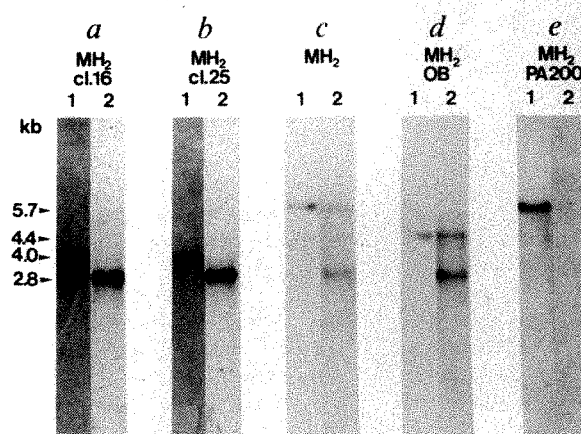


Fig. 3 Size of viral RNA in MH2 wild-type or mutant infected chicken fibroblasts and NR cells. Northern blots of polyadenylated RNA were probed in lanes 1 with a 1.1-kb *Bam*H1-*Hpa*I *v-mil*-specific fragment (see Fig. 1c) and in lanes 2 with a 0.6-kb *Aha*I/*Hae*III-*Eco*RI fragment derived from the last exon of the chicken *c-myc* locus²⁴. The probes were labelled with ³²P by nick translation and used as described previously¹¹. Viruses used are listed across the top of the figure. Lanes a-d, transformed fibroblasts; lane e, MH2-PA200-infected NR cells. Transformed fibroblasts in lane c were obtained after transfection with a molecularly cloned MH2 provirus (pMH2-Hd)¹ together with a plasmid containing a biologically active RAV-1 provirus (a generous gift from Dr J. M. Bishop).

tion between wild-type MH2 and its helper virus, resulting in the probable substitution of $\Delta\text{gag-pol-}\Delta\text{env}$ sequences of the helper by $\Delta\text{gag-mil-}\Delta\text{myc}$ sequences from wild-type MH2 (to be published elsewhere).

MH2-PA200 and MH2-LI200 were as efficient as wild-type virus in inducing proliferation of NR cells (Fig. 1b). However, in contrast to wild-type virus-infected cells, NR cells infected with either mutant were not morphologically transformed nor were they able to form colonies in soft agar-containing medium. Both virus mutants also failed to transform macrophages (T. Graf, personal communication) and chicken embryo fibroblasts (not shown). The latter is surprising since the mouse virus MSV3611¹⁹ that contains *v-raf*, the mouse-derived counterpart of *v-mil*, readily transforms mouse fibroblasts. This may indicate a structural difference in the two transduced genes or a different response of the target cells in the chicken and mouse species. Finally, reconstitution experiments showed that proliferating NR cells infected with MH2-PA200 became rapidly transformed on superinfection with MC29 (not shown).

The results presented here show that the ability to induce proliferation and transformation of NR cells from 7-day-old chicken embryos is a characteristic property which distinguishes MH2 from the other *v-myc*-containing viruses. Characterization of mutants MH2-PA200 and MH2-LI200 demonstrates that expression of *v-mil* is both necessary and sufficient to account for the mitogenic property of MH2 in NR cells. However, infection of NR cells and fibroblasts with mutant viruses expressing only *v-mil* does not result in cell transformation. That the mutant MH2-LI200 exhibits similar biological properties indicates that the *v-mil* sequence of MH2-PA200 is similar to that of wild-type virus. The *v-mil* gene shares significant sequence homology with other oncogenes of the *src* gene family¹⁴ which induce both proliferation and transformation of NR cells^{5,15}. Interestingly, mutants in the *src* gene that, like *v-mil*, induce NR cell multiplication without transformation, have been described⁶. Several oncogenes of the *src* gene family encode plasma membrane-associated protein kinases specific for tyrosine residues, a property also shared by several growth factor receptors^{16,17}. In contrast, P100^{gag-mil} appears localized in the cytoplasm of transformed fibroblasts and is associated *in vitro* with a serine/threonine-specific protein kinase activity^{18,19}. Such differences in intracellular localization and/or kinase specificity

may explain the lack of transforming capacity of the *v-mil* product. Whether the mitogenic signals induced by the expression of *v-mil* and *v-src* in NR cells share common primary targets remains to be determined.

Mutants of MH2 expressing only the *v-myc* oncogene fail to induce transformation and proliferation of NR cells in the conditions used, although they still transform fibroblasts and macrophages. Therefore, the *v-myc* genes of MH2 and MC29 seem to be functionally indistinguishable, despite their structural differences^{20,21}. However, transformation of NR cells by the *v-myc* gene can be established in cells already infected with mutants expressing only the *v-mil* gene. These results indicate that transformation of chicken NR cells by MH2 requires cooperation of the two oncogenes.

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Telomeric reciprocal recombination as a possible mechanism for antigenic variation in trypanosomes

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In African trypanosomes, antigenic variation is achieved through differential gene activation, with one antigen gene being expressed at a time among a large collection of antigen-specific sequences. Transcription of the antigen gene always takes place in a telomere, but different telomeres can alternatively act as the expression site¹⁻⁴. Telomeric antigen genes can be expressed without apparent DNA rearrangement⁵⁻⁸, but they can also, like non-telomeric genes, have access to the telomeric expression site through a duplicative transposition mechanism resembling gene conversion⁷⁻¹⁰. We report here that, as previously suggested^{3,6}, telomeric genes may use another route to be activated. This mechanism of gene activation is by reciprocal crossing-over upstream from the gene, in the so-called 'barren' region. This allows the antigen gene to be placed in the previously activated telomere, while inactivating the formerly expressed gene by recombination into a silent environment. At least for the telomeric antigen gene described here, three possible activation mechanisms coexist.

In *Trypanosoma brucei*, antigen gene activation frequently involves DNA rearrangements, although occasionally telomeric antigen genes seem to be expressed without^{6,8,11} or with only minor^{3,7} concomitant changes. To explain this mode of gene activation, we suggested that telomeric genes could be placed in the expression site following reciprocal exchange with the previously expressed gene^{3,6}. However, in the clone derivation AnTat 1.1B→1.6C→1.1G, we observed that the antigen genes involved were expressed without apparent rearrangement, even at the chromosome level: the gene *1.1B*, carried by a 225-kilobase (kb) minichromosome, remains in the same location whether expressed (in clones AnTat 1.1B and 1.1G) or not (in clone AnTat 1.6C), while the *1.6* gene is always found in a large chromosome irrespective of whether it is active or inactive (M.G. and E.P., unpublished results). Similar observations have been made in other cases of gene activation without DNA duplication^{2,12} and suggest that at least some telomeric antigen genes can be activated *in situ*, although the nature of the activation mechanism remains unknown.

At the other extreme, telomeric antigen gene activation may involve important DNA rearrangements. This is true for the AnTat 1.1C→1.3B switch, where a telomere, carrying a 1.3-specific sequence, has converted another telomere over more than 40 kb³. Gene conversions of this size are unusual and are thought to result from a lack of homology between the interacting telomeres, so that the conversion can only be triggered far upstream from the gene. Therefore, we considered another switch involving the same two telomeres, but in reverse order, to check if it would again lead to a large conversion. This was achieved through cloning AnTat 1.10D from AnTat 1.3A (see Fig. 1 legend for details). Indeed, the two telomeres expected to be involved in this antigenic switch are the same as those involved in the AnTat 1.1C→1.3B clone derivation. These telomeres carry the *1.3* and *1.10* genes, respectively; the latter is a member of the *1.1* gene family, which was partially converted in the AnTat 1.1C clone^{3,10}. Surprisingly, we found that in the AnTat 1.10D clone, the *1.10* gene was activated without duplication, although its environment was completely altered. Restriction mapping and determination of the chromosomal location of the two telomeres concerned unambiguously revealed that the switch from AnTat 1.3A to 1.10D was associated with a reciprocal event in which the *1.10* gene recombined into the active *1.3A* telomere. In turn, the previously expressed gene (*1.3*) was found to have recombined into the old *1.10* gene environment, where it became inactivated.

In the AnTat 1.3A clone, the actively transcribed *1.3* gene is a copy (an 'expression-linked copy' or ELC) of the single *1.3*-specific sequence, the 'basic copy' (BC)⁹. Figure 1 shows the restriction map of the telomere harbouring the *1.3A* ELC and a map of the telomere carrying the silent *1.10* gene in the same clone, while Fig. 2 shows the chromosomal location of these two sequences in the AnTat 1.3A clone. As noted previously¹², the *1.10* gene is located on a large chromosome, like all the *1.1* gene family members; the *1.3A* ELC is transcribed from a small 225-kb chromosome, while the *1.3* BC is on a 1,000-kb chromosome (ref. 12 and Fig. 2).

In the AnTat 1.10D clone, the restriction maps of the 5' environment of these two sequences have become exchanged, the crossover point lying in a 4-10-kb region devoid of most restriction sites ('barren' region), usually found upstream from the trypanosome antigen genes¹³ (Fig. 1). Accordingly, on a chromosome-sized AnTat 1.10D DNA blot (Fig. 2), it is the *1.3A* ex-ELC which is present on a large chromosome, instead of the 225-kb one, while the *1.10* gene is now found on the 225-kb chromosome.

Figure 3 shows that the *1.10* gene is really the sequence transcribed in the AnTat 1.10D clone. The 2-kb *EcoRI* fragment characteristic of the *1.10* gene and 5' neighbourhood^{3,10} is hypersensitive to DNase I in isolated AnTat 1.10D nuclei (Fig. 3a, arrowhead), as are transcriptionally active genes in other trypanosome clones¹⁴ or other eukaryotes¹⁵.

This sequence was not duplicated in the AnTat 1.10D clone,

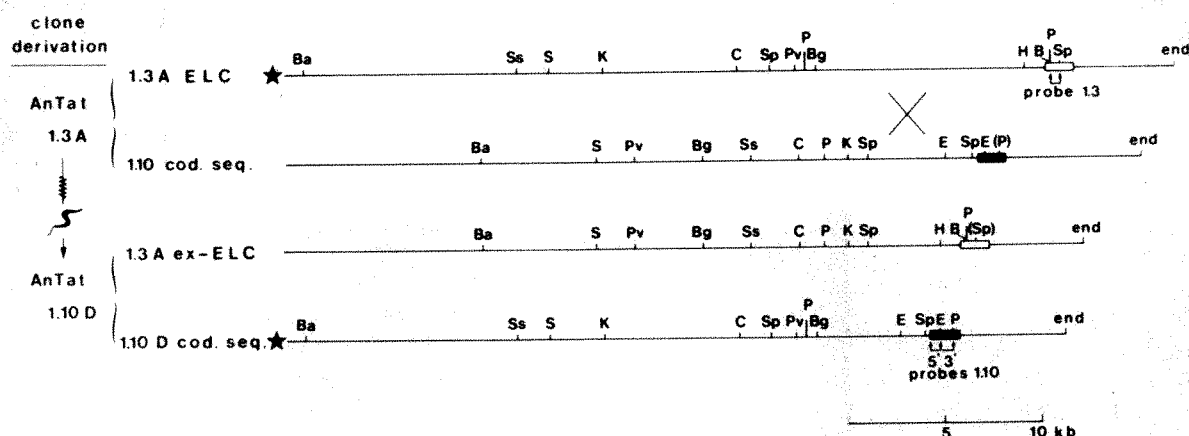


Fig. 1 Restriction maps of the telomeres carrying the *1.3* and *1.10* antigen genes, in clones AnTat 1.3A and 1.10D. ~, Antigenic variation; ~, cloning. In the restriction maps, the open and solid boxes represent the known extent of the *1.3* and *1.10* antigen genes, respectively, as deduced from complementary DNA analysis^{3,9,10}; the extent of the cDNA probes used to construct the maps by hybridization with Southern genomic blots²⁷ is delimited by arrows under the active genes. For each variant, the transcribed telomere is marked by a star. The bracketed sites are the targets for a telomeric DNA modification system, as discussed in the text. The crossover region between the two telomeres is indicated by a cross. B, *Bgl*I; Ba, *Bam*HI; Bg, *Bgl*III; C, *Cl*aI; E, *Eco*RI; H, *Hind*III; K, *Kpn*I; M, *Msp*I; P, *Pst*I; Pv, *Pvu*II; S, *Sal*I; Sp, *Sph*I; Ss, *Sst*I.

Methods. The *T. brucei* AnTat 1.10D clone was obtained by selecting an AnTat 1.10 expressor among the heterotypes arising in the cloned AnTat 1.3A population, passaged in mice at 2–7-day intervals (ref. 28; see ref. 9 for a description of the AnTat 1.3A clone). AnTat 1.10 variants were detected after 7 days in mice, and the cloning procedure took a total of 24 days.

as revealed, among many other restriction digests, by the *Pst*I and *Sph*I digestion patterns shown in Fig. 3b. The *Pst*I digestion allows the analysis of the 5' environment of the gene (see map in Fig. 1). The latter is usually contained in a 9-kb *Pst*I fragment^{3,10}, which is reduced to 7.5 kb in AnTat 1.10D DNA (Fig. 3b, arrowhead); no additional sequence is visible in that clone. A 16-kb *Pst*I fragment (arrow) is present in AnTat 1.3A DNA and lost in 1.10D; this is caused by incomplete cutting in the 3' *Pst*I site of the *1.10* gene (bracketed in the map of Fig. 1)¹⁶. Such a lack of cleavage is only found in silent telomeric sequences, and probably results from a special form of DNA modification affecting some restriction sites^{11,16,17}. Complete digestion at such sites is characteristic of actively transcribed antigen genes^{11,16,17}, and this is found to be true for the *1.10*

gene in AnTat 1.10D DNA. The *Sph*I digestion shown in Fig. 3b allows the analysis of the 3' environment of the gene (see Fig. 1). The latter is present in a telomeric fragment (arrowhead), whose size varies in different clones through chromosome 'growth' and shrinking^{18,19}. In blots of AnTat 1.10D DNA, the band containing this fragment is associated with a smear (asterisk). Such a smear is characteristic of actively transcribed sequences, presumably because terminal deletions are much more frequent in active, 'open', chromatin¹⁹. This again is in keeping with the *1.10* gene being expressed in the AnTat 1.10D clone.

Conversely, the *1.3A* ex-ELC is no longer expressed in the AnTat 1.10D clone. Indeed, *1.3*-specific RNA could not be found in Northern blots of that clone (not shown). Furthermore, the inactivation of the *1.3A* ELC can be revealed using the same three criteria as discussed above for *1.10* gene activation. The *1.3A* ELC, hypersensitive to DNase I in AnTat 1.3A nuclei⁹, is no longer preferentially digested in AnTat 1.10D (Fig. 4a, arrowhead). Moreover, this sequence is modified in AnTat 1.10D, as revealed by the *Pst*I + *Sph*I digestion pattern shown in Fig. 4b: a 3' *Sph*I site (bracketed in the *1.3* restriction map in Fig. 1) is incompletely cut in the ex-ELC (arrow) in the AnTat 1.10D clone, while this site was completely cut in the ELC in the AnTat 1.3A clone¹⁶. Finally, the smear associated with the transcriptionally active *1.3A* ELC in clone AnTat 1.3A (Fig. 4b, asterisk) is not present in the AnTat 1.10D DNA.

The results presented here strongly suggest that telomeric antigen genes can be activated (or inactivated) by reciprocal recombination taking place in the barren regions, upstream from the genes. Indeed, the telomere exchange precisely involves the genes active in the two successive clones. However, despite the short time elapsed in cloning the trypanosome variants, we cannot exclude the possibility of intermediates in which the *1.10* gene is activated independently of the recombination.

This mechanism of antigen gene activation has not been described previously, although it has already been proposed as a possible model for gene activation without duplication^{3,6,20}. It has been calculated that as little as 14 base pairs (bp) of homology are sufficient for homologous recombination in mammalian cells, although the recombination frequency decreases sharply under 200 bp of homology²¹. Since the barren telomeric sequences are presumed to be largely homologous and present near many antigen genes on numerous chromosomes^{2,13,22,23}, it

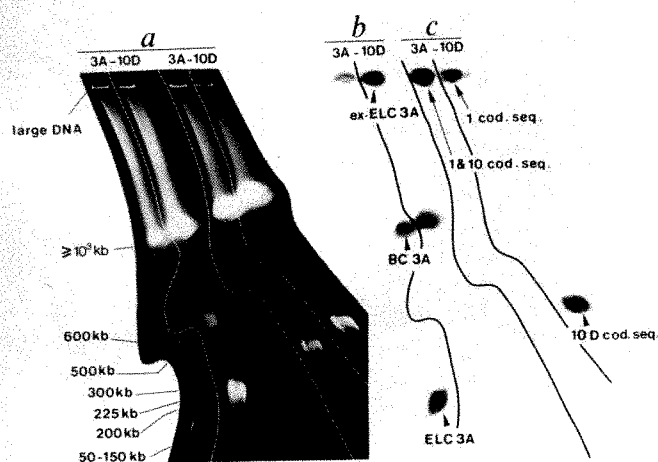


Fig. 2 Exchange of the *1.3* and *1.10* antigen genes in chromosome-sized DNA of the AnTat 1.3A and 1.10D clones. Chromosome-sized molecules of two trypanosome clones (indicated at the top of a, b and c) were separated using pulse-field gradient gel electrophoresis^{2,29}. The gel was stained with ethidium bromide (a) and then blotted onto nitrocellulose. The filter was hybridized with ³²P-labelled AnTat 1.3 (b) or 1.10 (c) cDNA probe (see Fig. 1). Compared with the size estimations reported in ref. 12, slight corrections have been incorporated here, according to new size estimates with a ladder of λ DNA polymers as marker (A. Bernards, personal communication).

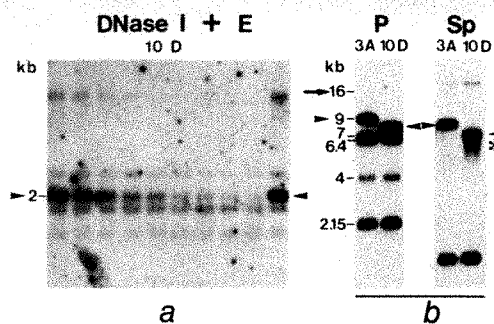


Fig. 3 The 1.10 gene is activated without duplication in the AnTat 1.10D clone. *a*, Kinetics of DNase I digestion in AnTat 1.10D nuclei, showing that the 1.10 gene (arrowhead) is selectively hydrolysed. Nuclei were isolated as described previously¹⁴, then incubated (0.5 mg DNA per ml) at 25 °C for 0, 30 s, 1, 2, 4, 8, 12, 16 and 30 min with DNase I (100 ng ml⁻¹), or 30 min without DNase I, from left to right lanes respectively. After *EcoRI* digestion and gel electrophoresis, the DNA was blotted onto nitrocellulose and hybridized with a 5' AnTat 1.10 probe (see Fig. 1). *b*, Restriction digests showing that, in the AnTat 1.10D clone, the 1.10 gene is not duplicated and bears two typical features of actively transcribed antigen genes (loss of telomeric DNA modification; terminal size heterogeneity). The *PstI* and *SphI* digestions generate fragments representing the 5' and 3' regions of the gene and its neighbourhood, respectively (see map in Fig. 1). The arrowhead and arrowed fragments are from the 1.10D gene, and are discussed in the text. The asterisk refers to the smear characteristic for active telomeres (see text).

is surprising that telomeric reciprocal recombination, such as the one described here, had not previously been detected, while, in contrast, gene conversion events are relatively frequent (reviewed in ref. 24). Why antigen gene switching by gene conversion is frequently observed, while reciprocal recombination events are not, is not known. In this context, it is perhaps significant that the reciprocal recombination event reported here has been detected between telomeres whose interaction was found, in a previous derivation, to lead to a very large conversion³, as if they were unable to convert each other at the 'usual' 5' end points of gene conversion, made up of 70-bp repeats^{13,22}. The reciprocal recombination observed here is apparently asymmetrical, since it was accompanied by a large deletion (~6 kb) in the 5' barren region of the 225-kb chromosome. This is consistent with the evidence^{13,22,23} that this region is at least partially made up of large arrays of 70-bp repeats, as recombination may thus take place at many possible sites. Size variation in the 5' barren region has also been observed linked to ELC insertion in the telomeric expression site^{3,4,7}.

As an example of genetic mechanisms involved in antigenic variation, expression of the AnTat 1.10 antigenic type is particularly interesting. This antigen is encoded by a telomeric gene, belonging to the AnTat 1.1 multigene family¹⁰, which can be transcribed following either of the two different gene activation procedures described to date. Indeed, this gene was found to be activated by ELC synthesis (that is, gene conversion) in several clones (ref. 10 and E.P., unpublished results), although the extent of the conversion appeared different in each clone, from about 2 to 3.5 kb. On the other hand, the same sequence was activated *in situ*, without duplication or chromosomal translocation, in the AnTat 1.1C clone³. In this case, it was also (subsequently ?) partially converted into an AnTat 1.1 antigen-coding sequence. Finally, the present study has found that it is expressed following telomeric reciprocal recombination. There are thus, for the same gene, many mechanisms of activation. These results suggest that, as in yeast²⁵, the telomeres in trypanosomes may be 'hotspots' of multiple rearrangement processes. The high frequency of genetic exchange in telomeres may be due to both the presence of repeated sequences and single-strand nicks, and the close contact between telomeres in mitosis²⁶. These rearrangements could sometimes generate newly func-

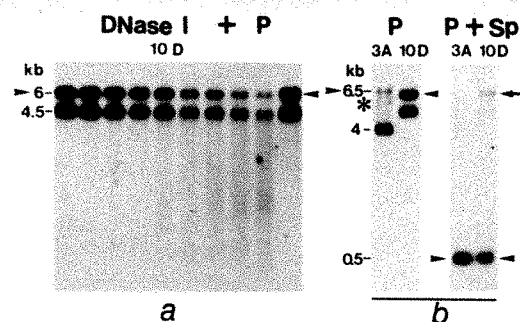


Fig. 4 The 1.3A ELC is conserved, but inactive, in the AnTat 1.10D clone. *a*, Kinetics of DNase I digestion in AnTat 1.10D nuclei, showing that the 1.3A ex-ELC (arrowhead) is no longer selectively hydrolysed. The order is the same as for Fig. 3a, except that the digestion was by *PstI*, and hybridization with the AnTat 1.3 probe. *b*, Restriction digests showing that, in the AnTat 1.10D clone, the 1.3A ex-ELC no longer exhibits the characteristic features of an actively transcribed telomeric sequence (namely a terminal smear and the absence of 3' restriction site polymorphism; see text). The *PstI* digestion allows the identification of the fragment extending to the telomere end (see map in Fig. 1). In AnTat 1.10D DNA, it is no longer associated with a smear, as it was in AnTat 1.3A DNA (asterisk). The *PstI* + *SphI* digestion reveals a fragment (arrow) due to a modification in the 3' *SphI* site of the 1.3A ex-ELC in AnTat 1.10D DNA; this polymorphism was not detected for the 1.3A ELC. Arrowheads indicate AnTat 1.3 gene-containing fragments.

tional antigen genes whose expression may be selected provided that it ensures adequate antigenic variation.

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(W2090)A

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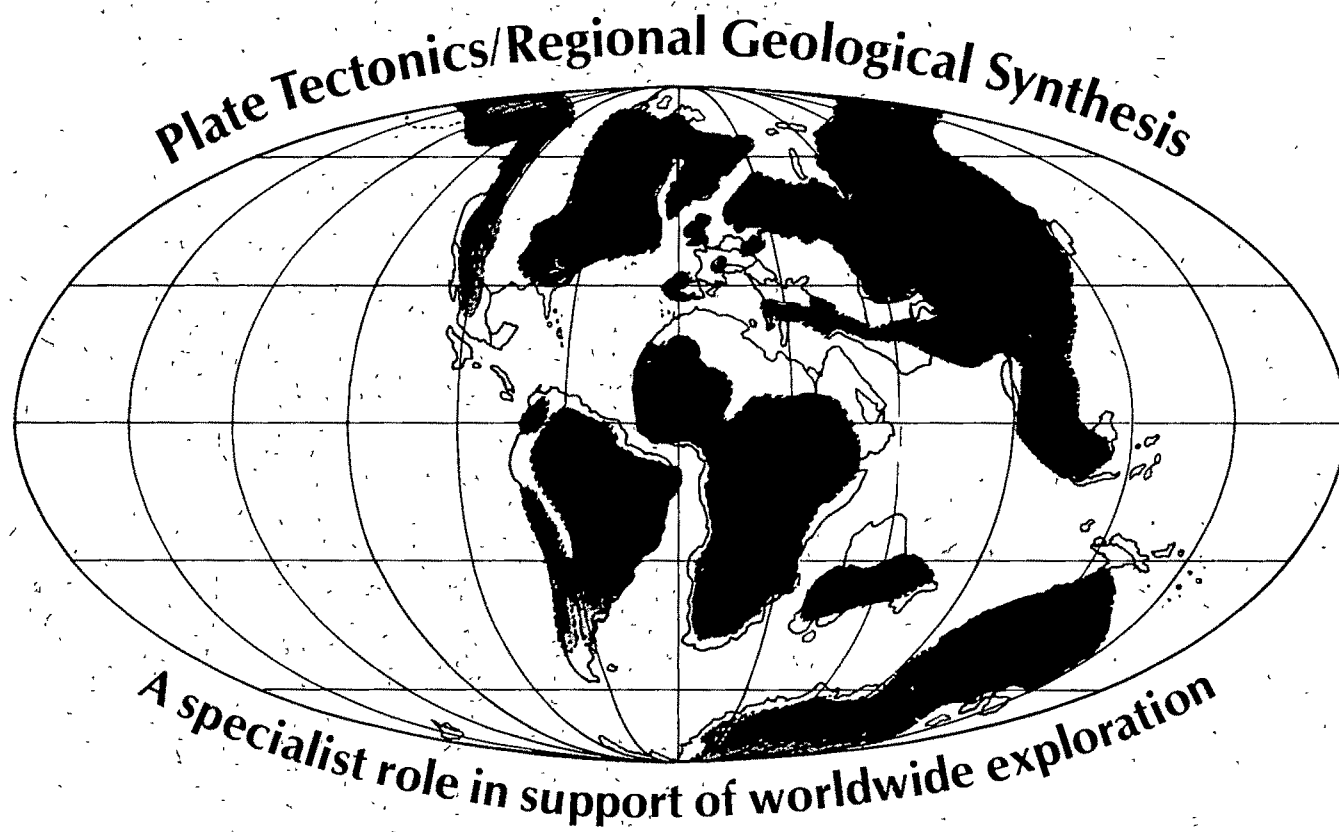
To apply send current resume and the names, addresses and telephone numbers of at least five (5) references to Robert L Pecsok, Dean of Natural Sciences, 235 Bilger Hall, 2545 The Mall, University of Hawaii, Honolulu, HI 96822. Telephone: (808) 948-6451.

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- 1 Protein structure and function, or
- 2 Eukaryotic molecular genetics; somatic cell genetics.

Applicants should have a doctoral degree with at least 3 years of post-doctoral experience, and each is expected to carry out an independent active research program. Teaching responsibilities include participation in courses in Biochemistry, genetics, and developmental biology for medical students and PhD candidates. Compensation will depend on professional experience and will conform with institutional standards. Potential for collaboration exists with other departments of the University in a growing research environment that includes the Oregon Regional Primate Research Center, the Portland Shriners Hospital Research Unit and a new Institute for Advanced Biomedical Research. Co-chairmen of the search committee are Richard T. Jones, Chair, Department of Medical Genetics. Applicants should send curriculum vitae, bibliography, reprints of representative papers, a summary of career goals and research plans, and names of three references to: Biochemical Genetics Search Committee, School of Medicine, L224, Oregon Health Sciences University, 3181 Southwest Sam Jackson Park Road, Portland, Oregon 97201. All applications received by September 15, 1985 will be considered. Women and minority candidates are especially invited to apply. OHSU is an Equal Opportunity/Affirmative Action Employer. (NW132)A

THE UNIVERSITY OF NEW ENGLAND
Equal Opportunity is University Policy
DEPARTMENT OF BIOCHEMISTRY, MICROBIOLOGY & NUTRITION
Lecturer in Human Nutrition

The appointee should hold a postgraduate degree in the field of human nutrition and preferably a qualification in dietetics as well.

The appointee will be expected to teach and develop courses in human nutrition, to supervise honours and postgraduate students and carry out research

The Department is well equipped for research in a number of areas, including whole body calorimetry and energy metabolism of man

Informal enquiries to Associate Professor R.B. Cumming (067) 73-2510 or ISD (61 67 73-2510).

SALARY:	A\$26,236 — A\$34,467 per annum
CLOSING DATE:	30th August 1985
POSITION NO:	517

The appointment will be to the permanent staff but the University reserves the right to make the appointment probationary where it considers this appropriate.

Other conditions include superannuation, assistance with travel and removal expenses and with buying or building a house in Armidale

Applications including the names and addresses of three referees and stating the position number should be sent to the Staff Officer, University of New England, Armidale N.S.W. 2351, prior to the closing date. Applicants should forward a copy of this advertisement to their referees and ask them to send their reports direct to the Staff Officer, Mr B.G. Turner, marking their envelopes 'Private and Confidential' and quoting the position number, before the above closing date so as to expedite the appointment process. (W2095)A

**POST DOCTORAL
RESEARCH POSITION**

available immediately for a minimum of three years to study Cell Surface Phenotype, DNA Content and Kinetic Properties of Lymphoma cells using the flow cytometer as the main analytical instrument. Independent research in related areas such as Lymphoma Growth Regulation and Differentiation, and Anti Idiotypic Antibody Production will be encouraged.

At least two years experience in cell biology and/or cellular immunology is required. Stipend commensurate with experience.

Please send a CV and names of references to: Paul C. Braylan, M.D., Professor, Department of Pathology, Box J-275, JHMHC College of Medicine, University of Florida, Gainesville, Florida 32610. (NW130)A

**M.R.C. CLINICAL RESEARCH CENTRE
(NORTHWICK PARK HOSPITAL)
WATFORD ROAD, HARROW
MIDDXX, HA1 3UJ**



NON-CLINICAL SCIENTIST

Applications are invited from biochemists with an interest in genetics for a postdoctoral position in the division of immunochemical genetics for investigations on glycosyltransferase enzymes involved in the biosynthesis of carbohydrate antigens.

The project involves purification of the relevant enzymes and a study of their properties and structure in preparation for molecular genetic studies on the relevant genes. Applicants should have experience in enzyme purification and protein chemistry.

The appointment is for 3 years starting as soon as possible. The salary will depend on qualifications and experience and will be within the range £8,920 — £14,925 pa plus £1,233 pa London Weighting.

Applications together with curriculum vitae, research experience and the names of two professional referees should be sent to Dr Winifred M Watkins, Division of Immunochemical Genetics not later than 29th August 1985 (172071)A

CSIRO
AUSTRALIA
**APPLIED ELECTRON
MICROSCOPY**

\$26,001 — \$38,243

**DIVISION OF CHEMICAL PHYSICS
CLAYTON VIC**

CSIRO conducts scientific and technological research in laboratories located throughout Australia and employs about 7,500 staff, of whom some 2,900 are professional scientists. The Organization's research activities are grouped into five Institutes: Animal and Food Sciences, Biological Resources, Energy and Earth Resources, Industrial Technology and Physical Sciences. The CSIRO Division of Chemical Physics is a member of the Institute of Physical Sciences.

GENERAL: The Division of Chemical Physics is principally located at Clayton, Victoria, with a smaller unit at the Lucas Heights Research Laboratories, Lucas Heights, New South Wales. The Division prosecutes a broad program of research in chemical physics, using fundamental methods. Presently there are twelve sections: atomic, mass and molecular spectroscopy, applied optics, electron microscopy, electron diffraction, X-ray diffraction, computing, theoretical physics, crystal defect physics, radiation effects (including radiation chemistry and ion implantation), solid state science, surface science, and instruments and engineering services. Divisional research objectives are both fundamental and applied.

BACKGROUND: Research programme aims and objectives at the Division of Chemical Physics are constantly subject to reassessment. Two sections — electron microscopy and electron diffraction — will shortly be consolidated into a single group. All research and development of the electron microscope as an instrument 'per se' will be phased out and, while some fundamental research into electron scattering and associated problems will continue, there will be a growing emphasis on applying the techniques of both transmission and scanning electron microscopes to materials problems specific to Australian Industry.

DUTIES: The Division currently conducts research into the surface and bulk properties of materials using electron microscopy and diffraction. The appointee will use these techniques to investigate how ion implantation and similar methods modify, or "tailor" such properties. Access to modern high speed computing facilities for the quantitative analysis of high resolution electron micrographs is available. The appointee will also participate in a broadening of the present ion implantation and surface modification programs to include metals, alloys, insulators, glasses, polymers and ceramics. High resolution electron microscopy as well as conventional diffraction and scanning electron microscopy techniques will be used in materials analysis and characterisation. Encouragement will be given to develop micro-analytical techniques based on dynamical effects which occur in the inelastic scattering of fast electrons.

QUALIFICATIONS: A PhD degree or equivalent, with experience in modern electron microscopy and diffraction techniques. A background in Metallurgy and Materials Science will be an advantage.

Above all, the successful candidate will have a positive and aggressive attitude towards identification and solution, using a variety of electron scattering techniques, of the materials problems of Australian industry.

TENURE: Indefinite with Australian Government superannuation benefits available.

APPLICATIONS: Stating relevant personal particulars, including details of qualifications and experience, the names of at least two professional referees and quoting reference No A1663, should be directed to.

The Chief
 CSIRO Division of Chemical Physics
 PO Box 160
 CLAYTON VIC 3168

by 4 weeks from date of issue

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER

(W2107)A

National Physical Laboratory

Theoretical Physicist

A small theoretical studies group is being established within this Laboratory to advance its understanding of the interaction of radiation with matter and to apply theoretical methods in support of the standards programme

A theoretical physicist is required to join this group to work on problems associated with high energy photon and electron absorbed dose determinations and conservation of exposure measurements to absorbed dose. You will be expected to initiate and manage projects within the overall framework of the branch programme and in collaboration with other groups. You will have access to the Laboratory's extensive irradiation and computing facilities

You must have a good honours degree or equivalent qualification in an appropriate subject whilst a higher degree in radiation physics is desirable. You should also have a proven ability to tackle complex theoretical problems at post-doctoral level

Salary: £12,900-£17,005 (including £545 Outer London Weighting) with starting salary according to qualifications and experience.

RELOCATION EXPENSES UP TO £5000 MAY BE AVAILABLE.

For further details and an application form (to be returned by 30 August 1985) write to Civil Service Commission, Alencon Link, Basingstoke, Hants RG21 1JB, or telephone Basingstoke (0256) 468551 (answering service operates outside office hours).

Please quote ref: S(G)677.

(7373)A

Scientific
Civil Service

Department of Trade and Industry

The Civil Service is an equal opportunity employer

THE HATFIELD POLYTECHNIC SCHOOL OF NATURAL SCIENCES RESEARCH ASSISTANT

£5,181 — £5,910 plus £264 local weighting

We have a vacancy for a well qualified Research Assistant in the field of Molecular Biology of chloroplast DNA replications. Please send CV and two references to Dr S A Boffey, Division of Biological & Environmental Sciences, The Hatfield Polytechnic, PO Box 109, Hatfield, Herts, AL10 9AB.

Please quote reference: 982.

(7325)A

CSIRO

AUSTRALIA

MOLECULAR GENETICIST

\$26,001 — \$38,243

DIVISION OF MOLECULAR BIOLOGY
NORTH RYDE NSW

CSIRO conducts scientific and technological research in laboratories located throughout Australia and employs about 7,500 staff, of whom some 2,900 are professional scientists. The Organization's research activities are grouped into five Institutes: Animal and Food Sciences, Biological Resources, Energy and Earth Resources, Industrial Technology and Physical Sciences. The CSIRO Division of Molecular Biology is a member of the Institute of Animal and Food Sciences.

GENERAL: The Division of Molecular Biology conducts scientific research in molecular genetics and cell biology. It has a staff of approximately 100. Programs are divided into: Tissue Growth and Development, DNA Maintenance and Modification and Gene Technology. It is situated in Sydney, at North Ryde, close to Macquarie University and major biotechnology companies.

DUTIES: As a member of a team working on several aspects of DNA maintenance and modification, the appointee will conduct a program of research on the mechanisms of mutagenesis and antimutagenesis in bacteria. This work will be directed towards understanding the role cellular processes play in the modulation by certain chemical agents of mutagenic activity.

QUALIFICATIONS: A PhD degree with training in microbial genetics, supported by established research ability, preferably with mutagenicity assay systems in *Escherichia coli* and *Salmonella typhimurium*. Experience in manipulating genes in bacteria using conjugation transduction and transposon techniques, experience in genetic toxicology and/or familiarity with techniques used in detecting DNA-damage and mutagenicity would be of considerable advantage.

TENURE: Indefinite with Australian Government superannuation benefits available.

APPLICATIONS: Stating relevant personal particulars, including details of qualifications and experience, the names of at least two professional referees and quoting reference No. A6705, should be directed to:

The Chief
CSIRO Division of Molecular Biology
PO Box 184
NORTH RYDE NSW 2113.

by 4 weeks from date of issue

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER

(W2109)A

POST-DOCTORAL FELLOW OR PROFESSIONAL RESEARCH ASSOCIATE

Applications are invited for a position as post-doctoral fellow or research associate to work on a study of the molecular mechanisms that control the expression of Gonadotropin releasing hormone.

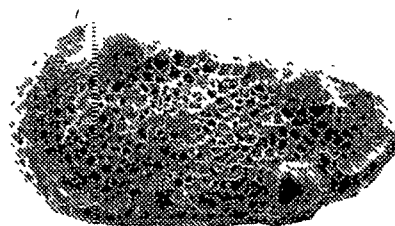
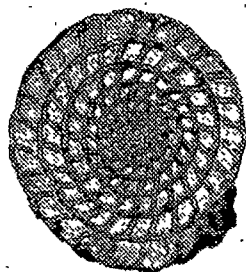
In accordance with Canadian immigration requirements priority will be given to Canadian citizens and permanent residents.

Please send current curriculum vitae and two letters of recommendation to:

OR

Vikram Misra
Dept of Veterinary Microbiology
WCVM
University of Saskatchewan
Saskatoon, Saskatchewan
S7N 0W0
(306) 966-7218

Bruce Murphy
Dept of Biology
University of Saskatchewan
Saskatoon, Saskatchewan
S7N 0W0
(306) 966-4411
(NW113)A



Senior Micropalaeontologist

A challenging career in biostratigraphy

This is an opportunity for a micropalaeontologist with at least 5 years' post-doctoral experience in industry, government or academia.

Your responsibilities will be many and varied. They will include analysing individual wells as they are being drilled, sometimes at the well site; analysing groups of wells from individual oil fields or entire petroleum provinces to establish ages, correlations and palaeoenvironments. These studies can help to improve seismic interpretations and our understanding of reservoir geometry.

There are also opportunities for involvement in multi-disciplinary groups working on operational problems or on ad hoc research and development.

Our research is directed to building the industrial biostratigraphy of tomorrow and skills and interests in computer manipulation of biostratigraphic data

will, therefore, be an advantage.

You will work with BP's explorers worldwide, and you can expect short or long term postings to operating centres such as Aberdeen, Canton or Holland at some time in your career. To help your interactions with exploration geologists, we offer a comprehensive training programme.

An attractive salary package is offered together with a non-contributory pension scheme. Excellent benefits include assistance with relocation, where appropriate.

Please write or telephone for an application form, quoting reference **SR 2629**, to: Anne Sheppard, BP Research Centre, Chertsey Road, Sunbury-on-Thames, Middx. TW16 7LN. Tel: Sunbury-on-Thames (09327) 62028.

BP is an equal opportunity employer.

(7370)A

BP

Research
SUNBURY-ON-THAMES

ASSISTANT/ASSOCIATE PROFESSOR IN IMMUNOLOGY

The Department of Microbiology

invites applications for a faculty appointment at the Medical College of Ohio. Candidates must have a PhD or MD at least 2 years of postdoctoral experience, and well-developed research goals. Individuals with interest in molecular-genetic approaches are particularly encouraged to apply, though others will be considered. The position represents a 12 month tenure-track appointment and involves the pursuit of an independent research program, participation in teaching graduate, medical, and nursing students, and participation in departmental and college activities such as the seminar programs.

Send curriculum vitae, names of three references, and a 2-3 page statement of research goals to: Dr Earl H Freimer, Chairman, Department of Microbiology, Medical College of Ohio, CS 10008, Toledo, Oh 43699.

Equal Opportunity Employer

(NW89)A

POSTDOCTORAL POSITION

HUMAN MITOCHONDRIAL GENETICS AND MOLECULAR BIOLOGY

Positions are available at Emory Medical School to study the molecular biology and genetics of the human mitochondrion and the role of mitochondrial genes in human evolution, genetic disease, and cancer. Mitochondrial genes located in both the nuclear and mitochondrial DNAs are being examined by approaches including gene cloning, DNA sequencing, restriction site variation, cell fusion, cell fractionation, and gene transfer. Interested individuals should send curriculum vitae and names of three referees to:

Douglas C Wallace, PhD
Professor of Biochemistry
Woodruff Memorial Building
Emory University School of Medicine
Atlanta, Georgia 30322

(NW123)A

CSIRO
AUSTRALIA
RESEARCH LEADER —
TROPICAL RAINFOREST
ECOLOGY
\$39,692 — \$52,212
DIVISION OF FOREST RESEARCH
TROPICAL RAINFOREST
RESEARCH CENTRE
ATHERTON QLD

CSIRO conducts scientific and technological research in laboratories located throughout Australia and employs about 7,500 staff, of whom some 2,900 are professional scientists. The Organization's research activities are grouped into five Institutes: Animal and Food Sciences, Biological Resources, Energy and Earth Resources, Industrial Technology and Physical Sciences. The CSIRO Division of Forest Research is a member of the Institute of Biological Resources.

A highly experienced scientist is required to lead expanding research activities at the CSIRO Tropical Rainforest Research Centre, Atherton, Queensland and co-ordinate associated projects by scientists based at other locations.

Rainforests in Northern Queensland extend in a discontinuous belt for about 500 km along the eastern coast from just north of Townsville to Cooktown. They occur on a wide range of soil types from sea level to over 1500 m elevation. Rainfall varies from more than 4000 mm to less than 1000 mm some 100 km inland. Atherton itself is at an elevation of 750 m, has a mean temperature of 20°C and an average rainfall of 1400 mm.

Research staff at the CSIRO Laboratory in Atherton are from the Division of Forest Research (6 scientists), Wildlife and Rangelands Research (1 scientist) and Plant Industry (1 scientist). Total staff complement at present is 22. The Headquarters of the *Division of Forest Research* are at Canberra ACT; research is also undertaken from laboratories in Perth, Mt Gambier, Hobart and Brisbane, as well as Atherton. Research in progress at Atherton is in the programs Soils and Nutrition, Genetic Resources and Breeding Strategy, and Ecophysiology. The Division of Plant Industry maintains at Atherton, as part of the Australian National Herbarium, a major collection of rainforest specimens. The *Divi-*

sion of Wildlife and Rangelands Research work is on the response of animal populations to changes in the forest, and on the role of vertebrates in regeneration and succession. It is expected that, with the new developments, total staff involved in the tropical rainforest program, at Atherton and elsewhere, will grow to about 40.

DUTIES: The appointee will lead a major new thrust in rainforest research and will have a high level of responsibility for planning, development, co-ordination and oversight of the overall program. The work will be done by the staff at Atherton and by groups based elsewhere, including other CSIRO Divisions. These are likely to include modellers and ecologists from the Division of Water and Land Resources, wildlife ecologists from the Division of Wildlife and Rangelands Research, plant taxonomists from the Division of Plant Industry, insect taxonomists from the Division of Entomology; and geneticists at the Division of Forest Research. The leader will also be responsible for the efficient operation of the Atherton laboratory including personnel and financial management, for developing a program of visiting scientists and related seminars and for fostering interaction with agencies such as the Queensland Forest Department, Queensland National Parks and Wildlife, and University groups.

While the initial focus will be on tropical rainforests, the interests could eventually extend to other tropical forests. CSIRO also has a program on open forests in the wet/dry tropics based at Darwin, under separate leadership.

The appointee will also initiate and be responsible for his/her own research programs within the tropical rainforest field.

QUALIFICATIONS: A PhD degree or equivalent qualifications in a relevant field, together with a record of substantial research experience and achievement. Demonstrated capacity in the management of research work and staff is essential. Appointment at a higher level would be considered for a suitably qualified applicant.

TENURE: Indefinite with Australian Government superannuation benefits available.

APPLICATIONS: Stating relevant personal particulars, including details of qualifications and experience, the names of at least two professional referees and quoting reference No. A2739, should be directed to:

The Chief
 CSIRO Division of Forest Research
 PO Box 4008
 QUEEN VICTORIA TERRACE ACT 2600
 by 4 weeks from date of issue

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER
 (W2111)A

ELECTRICAL ENGINEER/COMPUTER SCIENTIST
THE UNIVERSITY OF GEORGIA

is establishing in October 1985, the Complex Carbohydrate Research Center. The CCRC is particularly well-equipped and well-organized for collaborative research. The CCRC will start with five faculty and about 25 members and is projected to increase in 3 or 4 years to about 15 faculty and 100 members. The CCRC will be equipped with nuclear magnetic resonance spectrometers, several mass spectrometers, and many micro and mini-computers, which will interface with the University's Cyber super-computer. The CCRC wishes to hire an individual with experience in testing and tuning high frequency measuring instruments (eg ultra-high field NMR and high-mass mass spectrometry) thorough knowledge of analog and digital electronic techniques and experience in programming (in assembly as well as higher level languages) using micro-, mini-, and/or super-mini computers. The individual will use computers for such laboratory needs as system control and digital signal processing, eg fast Fourier processing. This individual's primary responsibility will be to maintain (and modify) the laboratory's instruments. The successful applicant will have an interest in interacting with biologists and chemists. Annual salary (about \$40,000) will depend on training and experience. Applications will be accepted for an indefinite period, although evaluation of those applications received will begin by October 1, 1985. Applications should be sent to:

Dr Peter Albersheim, Director, Complex Carbohydrate Research Center, Richard B Russell Agricultural Research Center, PO Box 5677, Athens, Georgia 30613, USA.

The University of Georgia is an Affirmative Action/Equal Opportunity Employer (NW125)A

Faculty Positions available at Assistant, Associate and Full Professor levels in the newly established Center for Biotechnology. The Center consists of three divisions: (i) Hybridoma Technology; (ii) Recombinant DNA Technology and (iii) Cellular and Molecular Neurobiology.

Applicants should have research background in one of these areas. Experience and interest in biotechnological applications are desirable but not required.

Please send résumé, a brief description of research experience and future goals, and names of several references to:

Dr. Dominic Man-Kit Lam, Director



The Center for Biotechnology
Cullen Eye Institute
Houston, Texas 77030

(NW126)A

An Equal Opportunity Affirmative/Action Employer.

CANADA DIRECTOR OF RESEARCH

The Hospital for Sick Children, Toronto, Canada is seeking a leading scientist, M.D. or Ph.D., as Director of its Research Institute.

The Hospital for Sick Children is the largest paediatric hospital in North America and is the Department of Paediatrics, Faculty of Medicine of the University of Toronto. Located in central Toronto in close proximity to the University and other major teaching hospitals. Medical Staff and Investigators hold appointments at the University.

The Research Institute is a broadly based organization with an international reputation in medical research. A staff of 743 includes 70 full-time and 80 part-time investigators, 143 post and predoctoral fellows and full support services with a budget of \$25 million per annum.

Evidence of leadership capabilities and demonstrated excellence in a specific area of research are essential requirements for this position.

Salary and benefit program are negotiable.

Nominations or applications which should include a curriculum vitae should be sent to:

Mr. J. Douglas Snedden
President
The Hospital for Sick
Children
555 University Avenue
Toronto, Ontario, Canada
M5G 1X8 (NW131)A



SCIENTIST – DIVISION OF BLOOD PRODUCTS

£9,213 - £12,438

We require a scientist with a PhD in biochemistry, pharmacology, or the biological sciences, preferably with some post doctoral experience, to work in the Division of Blood Products.

The person appointed will be responsible for quality control tests on certain blood products. There is considerable scope for individual and collaborative research within the field of haemostasis, both locally and internationally.

The appointment is for 5 years, in the first instance. Salary is on a scale of £9,213 - £12,438 inclusive, dependent on age, qualifications, and experience.

For further details, please contact the Personnel Office, NIBSC, Holly Hill, Hampstead, London NW3 6RB. Tel. No. 01-435-2232. (7358)A

Please quote reference number: BL/035

NIBSC

National Institute for Biological Standards and Control

NOVO

Organic Chemist Pharmaceutical Research

Due to expansion of our pharmaceutical research, our Laboratory of Medicinal Chemistry has an opening for an organic chemist, ideally with post-doctoral experience within pharmaceutical or biochemical research.

The candidate must have good practical experience in synthetic organic chemistry, or have worked with isolation and identification of natural products, and be familiar with analytical and purification techniques used in organic chemistry.

The applicant will join an independently working research group consisting of chemists, biochemists, and pharmacologists.

The applicant is expected to engage her/himself with energy and enthusiasm in solving problems concerning her/his research and drug development.

Finally the applicant will be expected to participate in a continual development of a progressive and creative research setting which will give her/him the opportunity for a personal and professional development.

Our main research interest are focused on peptide hormones, neurotropics and new drugs in the inflammation area.

Applications marked "Organic Chemist" should be addressed to Personnel Dept. by 15th of September, 1985.

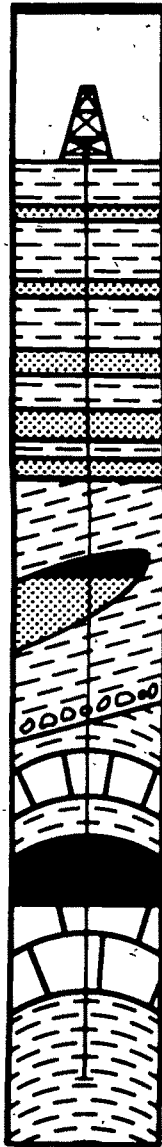
For further information please contact Claus Bræstrup, phone 45 2 98 23 33, ext. 2106 or B.F. Lundt; phone 45 2 98 23 33, ext. 2213.

NOVO INDUSTRIES

Novo Allé
DK-2880 Bagsvaerd
Copenhagen



Novo is a Danish pharmaceutical and biochemical Group whose products — primarily insulin and enzymes for industrial uses — are marketed throughout the world. The Novo Group has subsidiaries and information offices in 25 countries and employs approximately 4,600 people, of which approx. 900 in research and development, quality control and technological services. (W2114)A



Senior Stratigrapher/ Biostratigrapher

**"to work in support of
exploration and production"**

You will join a multi-disciplinary research group, whose goal is to develop new ideas and approaches to improve BP's use of biostratigraphy in support of our exploration and production activities. Our aim is to widen the perspective of biostratigraphic thinking beyond the traditional dating of sediments and interpretation of their paleoenvironment.

Our research interests cover such diverse topics as:

- paleobathymetry for subsidence analysis
- sealevel and its role in developing sedimentary sequences
- sequence stratigraphy and the relation between microfossils and stratigraphic sequences
- quantitative methods in biostratigraphy to improve correlation
- paleobiogeography to improve regional biostratigraphy

This challenging post calls for an unusual blend of geological skills. You should have a PhD, 8 years' post-doctoral experience, preferably gained in the petroleum industry, together with experience in sedimentology, seismic stratigraphy and biostratigraphy. Familiarity with the use of computers is essential.

An attractive remuneration package includes a non-contributory pension scheme, subsidised restaurant, on-site sports and social facilities and relocation assistance, where appropriate.

Please telephone for an application form or write enclosing a c.v., quoting reference **SR 2628**, to: Anne Sheppard; The British Petroleum Company plc, BP Research Centre, Chertsey Road, Sunbury-on-Thames, Middlesex TW16 7LN. Tel: Sunbury-on-Thames (09327) 62028.

BP is an equal opportunity employer.

(7371)A

BP

Research
SUNBURY-ON-THAMES

L'UNIVERSITE DE LAUSANNE

**ouvre une inscription pour
la poursuite d'un poste de
PROFESSEUR ASSOCIÉ**

à l'Institut de Biologie animale
de la Faculté des sciences
avec entrée en fonction au 1^{er}
septembre 1986.

Le titulaire enseignera la virologie générale et moléculaire des étudiants en biologie de 2^{ème} cycle, et exercera son activité de recherche en virologie animale et moléculaire. Une large formation en biologie animale est souhaitée.

Le cahier des charges peut être obtenu auprès du Doyen de la Faculté des sciences.

Les candidatures (avec curriculum vitae, 3 références, liste des publications et tiré part des 5 publications les plus significatives) sont à adresser au professeur Bernard TEST, doyen de la Faculté des sciences, Collège, propédeutique, 1015 Lausanne (Suisse) avant le **31 octobre 1985**. (W2091)

THE OHIO STATE UNIVERSITY CHAIRPERSON AND PROFESSOR Department of Poultry Science

Candidates must hold a Ph.D. in Poultry Science or a closely related field; have a successful record in research and experience in teaching or extension; demonstrate leadership ability; interest in both basic and applied research; dedication to quality graduate and undergraduate education. Salary is negotiable and commensurate with qualifications and experience.

Send curriculum vitae and name and addresses of three references by September 30, 1985 to: Dr David Latshaw, 674 West Lane Ave Columbus, Ohio 43210.

Equal opportunity/affirmative action employer. (NW118)A

Rechercher
des médicaments
pour le « cerveau âgé »



Neuropharmacologue

Doctorat d'état + 1^{ère} expérience de recherche

Dans le cadre de ses nouvelles orientations de recherche, le Centre de Recherche Delalande recrute un neuropharmacologue.

Ce poste s'adresse à un pharmacien, médecin ou universitaire, âgé de 30-35 ans possédant un doctorat d'état en pharmacologie ainsi qu'une première expérience de recherche.

Intégré au sein du Département de Recherche Biologique, il aura à créer sa propre

équipe et à l'impliquer dans un projet de recherche d'un nouveau médicament des affections pathologiques liées au vieillissement. Il devra établir et développer des méthodologies pour évaluer les nouvelles molécules.

Imprégné des concepts de pharmacologie moléculaire, il participera au sein d'une équipe pluridisciplinaire à la conception de nouveaux programmes.

Veuillez adresser votre dossier de candidature à Monsieur LANGLOIS.

MEDEA BA

**Centre de Recherche
Delalande**

10, rue des Carrières - 92500 RUEIL-MALMAISON - FRANCE (W2093)A

CANCER RESEARCH CAMPAIGN CELL BIOLOGIST

PhD graduate required for research into effects of ionizing radiations on the cellular and subcellular level. Candidates should be familiar with mammalian cell culture and preferably have experience of assays of DNA damage and repair. Experience of biochemical and molecular biological techniques desirable. Salary on MRC Grade II Scientist scale (max. £12,438 pa inc Londo weighting). Apply with full CV and names and addresses of two referees to: Administrator (5), CRC Gray Laboratory, Mount Vernon Hospital, Northwood, Middx. HA 2RN. (73121)A

ANALYTICAL CHEMIST/ BIOCHEMIST

Dista Products Limited is part of Eli Lilly and Company, a leading U.S. Manufacturer of Pharmaceutical, Veterinary and Agrochemical Products.

The Company wishes to appoint an Analytical Chemist or Biochemist for its Analytical Technical Services Group. This group undertakes a wide range of analytical work, largely non-routine, and is currently highly involved in supporting the biosynthetic production of polypeptides including human insulin.

Applications are invited from Chemists or Biochemists with several years' experience in analytical work. A prime requirement is a working knowledge of analytical techniques as applied to proteins/polypeptides, particularly HPLC, SEC, RIA and ELISA. A Postgraduate qualification is desirable but not essential.

The Company offers excellent career opportunities to suitably motivated employees and an attractive salary will be offered. Other Company benefits include a non-contributory pension plan, membership of BUPA and flexible working hours. Relocation assistance in appropriate cases will be given.

Please apply, in writing, outlining academic and career history to:



The Personnel Manager,
Dista Products Limited
Fleming Road,
Liverpool L24 9LN
(7363)A

THE MEDICAL COLLEGE OF ST BARTHOLOMEW'S HOSPITAL DHSS DEPARTMENT OF TOXICOLOGY RESEARCH TECHNICIAN Salary Scale £6,822 - £9,180 incl

Applications are invited from technical staff, as well as recently qualified graduates, for this position to work on the biochemistry of the regulation of hepatic alpha-2u globulin synthesis.

The work will involve the techniques of cell culture, immunochemistry and molecular biology. Experience in these areas would be useful but is not essential since full training will be provided. The position is funded in the first instance by ICI PLC and will be for a period of one year.

Applications to: The Secretary, The Medical College of St Bartholomew's Hospital, West Smithfield, London EC1.
Closing date: 30 August 1985 (7362)A

CSIRO AUSTRALIA APPLIED/ COMPUTATIONAL MATHEMATICIAN

\$26,001 — \$38,243

DIVISION OF MATHEMATICS AND
STATISTICS
LINDFIELD NSW

CSIRO conducts scientific and technological research in laboratories located throughout Australia and employs about 7,500 staff, of whom some 2,900 are professional scientists. The Organization's research activities are grouped into five Institutes: Animal and Food Sciences, Biological Resources, Energy and Earth Resources, Industrial Technology and Physical Sciences. The CSIRO Division of Mathematics and Statistics is a member of the Institute of Physical Sciences.

The Division of Mathematics and Statistics has a staff of 95, including 72 professional scientists. The Division's Headquarters are at Canberra, and there are Regional Offices located at Melbourne, Sydney, Adelaide, Brisbane and Perth. Scientists of the Division provide advice on the statistical, mathematical and numerical aspects of research projects undertaken by scientists in other CSIRO Divisions, as well as conducting basic research in statistics and mathematics and working with Australian industry.

The Division has an active Applied and Computational Mathematics Program working on collaborative research projects with CSIRO Divisions in a variety of areas including manufacturing technology, oceanography, energy technology and applied physics. Members of the group are currently located in Canberra, Hobart, Melbourne and Sydney.

The appointee will be a member of the above Program and will be located in Lindfield and in addition to working on collaborative research projects with Sydney Divisions (including Applied Physics, Mineral Physics and Mineralogy and Radiophysics) will contribute to the basic research of some aspects of applied and computational mathematics, as well as to projects initiated with industry.

QUALIFICATIONS: A PhD degree or equivalent qualifications in a relevant branch of mathematics, together with a proven record of research and collaboration in relevant areas of mathematics. Expertise in the numerical solution of partial differential equations, numerical linear algebra and computational problem solving are particularly relevant. Familiarity with industrial applied mathematics and a commitment to the application of mathematics to practical and challenging industrial problems are also desirable.

TENURE: A term of 3 years, with the possibility of a further term. Australian Government superannuation benefits available.

APPLICATIONS: Stating relevant personal particulars, including details of qualifications and experience, the names of at least two professional referees and quoting reference No. M7743, should be directed to:

The Chief
CSIRO Division of Mathematics and Statistics
GPO Box 1965
CANBERRA ACT 2601

by 4 weeks from date of issue

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER
(W2108)A

Please mention **nature**

when replying to these advertisements

CSIRO

AUSTRALIA

STATISTICIAN

\$33,209 — \$45,083

DIVISION OF MATHEMATICS AND STATISTICS

GLEN OSMOND SA

CSIRO conducts scientific and technological research in laboratories located throughout Australia and employs about 7,500 staff, of whom some 2,900 are professional scientists. The Organization's research activities are grouped into five Institutes: Animal and Food Sciences, Biological Resources, Energy and Earth Resources, Industrial Technology and Physical Sciences. The CSIRO Division of Mathematics and Statistics is a member of the Institute of Physical Sciences.

The Division of Mathematics and Statistics has a staff of 95, including 72 professional scientists. The Division's Headquarters are at Canberra, and there are Regional Offices located at Melbourne, Sydney, Adelaide, Brisbane and Perth. Scientists of the Division provide advice on the statistical, mathematical and numerical aspects of research projects undertaken by scientists in other CSIRO Divisions, as well as conducting basic research in statistics and mathematics and working with Australian industry.

The Division has a new program called Statistics in Earth and Space Science. Within this program there are sub-programs to pursue statistical methodology in Crop Research, Earth Sciences, the analysis of spatial data and time series analysis.

DUTIES: As a senior member of the Crop Research Sub-program, the appointee will initiate collaborative research projects with other scientists involved in conducting field trials and will supervise a research project investigating statistical methodology in the design and analysis of field trials with particular attention to nearest neighbour methods.

QUALIFICATIONS: A PhD degree or equivalent qualifications in statistics, together with a proven record of research and collaboration in the design and analysis of experiments. Expertise in incomplete block and nearest neighbour methodology associated with the design and analysis of field trials is particularly relevant. The appointee should also have a demonstrated ability to supervise research in applied statistics and a commitment to the application of statistics to practical and challenging problems in the biological sciences.

TENURE: A term of 3 years, with Australian Government superannuation benefits available.

APPLICATIONS: Stating relevant personal particulars, including details of qualifications and experience, the names of at least two professional referees and quoting reference No. M7742, should be directed to:

The Chief
CSIRO Division of Mathematics and Statistics
GPO Box 1965
CANBERRA ACT 2601

by 4 weeks from date of issue

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER
(W2110)A

POST-DOCTORAL POSITION

available after 1 October

Cell biology-physiology-biochemistry laboratory studying isolated cardiac membrane channel proteins seeks

IMMUNOLOGIST OR PROTEIN BIOCHEMIST

with immunological experience for immunofluorescence and immunoelectron microscopic investigations. Applicant should have doctorate and experience with poly- and monoclonal antibody techniques.

Send curriculum vitae, description of research interests, references to: Dr. Ernest Page, Department of Medicine, BH Box 137, The University of Chicago, 5841 South Maryland Avenue, Chicago, Illinois 60637. Equal Opportunity/Affirmative Action Employer. (NW45)A

THE AUSTRALIAN NATIONAL UNIVERSITY

Applications are invited from suitable qualified men and women for appointment to the following position:

Faculty of Science

DEPARTMENT OF BOTANY LECTURER IN PLANT CELL BIOLOGY (FIXED TERM)

The appointee will contribute to teaching especially in second-year and third-year units in cell biology, may supervise honours and graduate students, will be expected and encouraged to conduct research, and to perform other administrative duties in the department. Applicants must have a broad interest in and knowledge of both biochemical and ultrastructural aspects of plant cell biology, should have relevant teaching experience and also should have engaged in productive research. The appointment is for the three years 1986-88 inclusive. The successful applicant will be expected to take up duty, if possible, in January 1986. Enquiries to the Head of Department (062)492866.

Closing date: 30 August 1985.
Ref: FS 18/7/1.

Conditions of appointment: Salary will be in accordance with qualifications and experience within the range: Lecturer \$A26,236 — \$34,467 pa.

Grants are provided towards travel and removal. Assistance towards housing is given to an appointee from outside Canberra. Eligible appointees will be required to join the Superannuation Scheme for Australian Universities. Maternity leave is available. The University reserves the right not to make an appointment or to make an appointment by invitation at any time. Applicants should quote the advertisement reference when requesting further particulars, and when submitting applications, which should be submitted to the Acting Registrar of the University, GPO Box 4, Canberra, ACT 2601, Australia. Further particulars also available from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

The University is an Equal Opportunity Employer. (W2103)A

THE UNIVERSITY OF NEW SOUTH WALES Sydney, Australia

LECTURER IN SCHOOL OF APPLIED GEOLOGY

(Tenure or Tenurable appointment)

(Ref: 1068)

A Lectureship in Geophysics will be available commencing January 1986. The successful applicant will be responsible for teaching and research in reflection and refraction seismic methods. Teaching will be at both undergraduate and postgraduate levels and will involve supervision of postgraduate research.

Applicants should have a PhD in Geophysics or equivalent research experience. A record of research ability and experience in reflection and refraction seismic methods is required. Preference will be given to candidates with experience in industry.

The School is dedicated to teaching and research in the application of fundamental geological and geophysical knowledge to problems of resource development. The appointee would be expected to maintain a similar attitude in his/her fields of interest.

Appointment will be either tenure or on the basis of a contract with provision for conversion to tenure.

Further information from Associate Professor P R Evans, Head of School, University of New South Wales, P O Box 1, Kensington NSW, Australia 2033 (tel: (02) 4268).

Applications close 20 September 1985.

Salary: \$A26,236 range \$A34,467. Commencing salary according to qualifications and experience.

For further information about conditions of appointment, method of application write to Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Equality of employment opportunity is University policy. (W2104).

POSTDOCTORAL RESEARCH FELLOW

with interest in tissue culture and application to the study of the inflammatory reaction, or

POSTDOCTORAL RESEARCH ASSOCIATE

with experience in the field.

Applications are invited for work to begin immediately. Salary based on MRC guidelines.

Send curriculum vitae and names of 2 referees to: Dr Henry Z Mo, Department of Pathology, Medical Sciences Building, University of Toronto, Toronto, Canada M5S 1A5.

RESEARCH ASSISTANT IN NEUROPHYSIOLOGY AND BEHAVIOUR

Applications are invited for this post to work on the activity of single neurones in visual and taste information processing in the primate brain. The post is appropriate for a postdoctoral scientist with experience in neurophysiology. The salary is on the RS 1A salary scale, £7,520 to £11,250.

Applications/enquiries in writing to Dr E T Rolls, University of Oxford, Department of Experimental Psychology, South Parks

UNIVERSITY OF
LIVERPOOL

Department of Medical
Cell Biology

SENIOR RESEARCH
ASSISTANT

Applications are invited for the above post-doctoral position to work with Dr S Marshall-Clarke on a project involving the identification and characterisation of growth and differentiation receptors on B lymphocytes.

The project is funded by the Wellcome Trust for a period of three years.

Ideally the applicant should have experience of cell culture, the production of monoclonal antibodies and the biochemical analysis of cell surface molecules.

Informal enquiries may be made to Dr S Marshall-Clarke, Tel 051 709 6022 Ext 2583.

Initial salary within the range £7,520 - £8,920 per annum (under review)

Applications, together with the names of three referees, should be received not later than 14 October, 1985, by The Registrar, The University, PO Box 147, Liverpool, L69 3BX, from whom further particulars may be obtained

Quote Ref: RV/177/N (7329)A

UNIVERSITY OF
NEWCASTLE UPON TYNE

DEPARTMENTS OF NEUROLOGY
AND PHARMACOLOGICAL
SCIENCES

POSTDOCTORAL
RESEARCH ASSOCIATE

Applications are invited for an MRC funded post to study defects of fatty oxidation in human skeletal muscle found in some metabolic diseases. Experience in enzymology or mitochondrial biology would be an advantage.

The appointment is available for three years, from 1st September 1985. Commencing salary up to £9,390 per annum on the Range 1A salary scale, according to age, qualifications and experience.

Candidates with a PhD or expecting to have gained a PhD by the Autumn should send an application with curriculum vitae with names and addresses of three referees to Dr D M Turnbull, Muscular Dystrophy Research Laboratories, Newcastle General Hospital, Westgate Road, Newcastle upon Tyne, NE4 6BE by not later than 31st August 1985. Further information may be obtained from Dr D M Turnbull or Dr H S A Sherratt (Telephone number (0632) 328511 Extension 3031).

Inorganic Chemist

£9,000 - £14,000

Amersham, Bucks

Amersham International plc is a highly successful and expanding UK Company, and a world leader in the supply of radiopharmaceutical products used in vital clinical diagnostics by hospital laboratories throughout the world.

Continued expansion of our R&D effort in radiopharmaceuticals has provided an excellent opportunity for a first class Inorganic Chemist to join a highly professional team pioneering new areas of interest for future product developments.

The post will ideally suit a graduate or postgraduate Inorganic Chemist, probably in their mid 20's, capable of demonstrating initiative, self-motivation and the flexibility to solve problems outside their specialist field. An interest in the synthesis and characterisation of novel transition metal compounds would be particularly relevant.

Salary to reflect ability will be within the range of £9,000 - £14,000 and is supported by an excellent benefits package including relocation assistance, where appropriate, to this pleasant South Bucks location.

Please write with full details to David Riley at the address below. Alternatively telephone Little Chalfont (02404)4488, ext. 3577 for an application form.

Amersham International plc,
Amersham Laboratories,
White Lion Road,
Amersham,
Bucks HP7 9NA.

(7369)A

Amersham

Intervet

**VETERINARY
VIROLOGIST**

A Virologist, preferably with a qualification in veterinary medicine, is required by Intervet Laboratories Ltd. The applicant would be required to participate in a programme of research into and the development of virus vaccines for domesticated animals.

Preference will be given to applicants in the age range of 25-35 years.

Salary in accordance with qualifications, age and experience.

Applications please to:
Mr W Baxendale
Intervet Laboratories Ltd
The Elms,
The Thicket,
Houghton,
Huntingdon
Cambridgeshire
PE17 2BQ.

172281A

Torry Research Station,
Aberdeen
Ministry of Agriculture, Fisheries and Food

Bacteriologist

... to research and advise on all aspects of the bacteriology of fish as food. You will be involved in practical problems in the processing industry where you will also have an advisory role. You will be expected to work as a member of a team on some projects and to develop individual research on others. Initially the individual research projects will be in the area of controlling the growth of bacteria in fishery products, which will involve studies into the interactions amongst controlling parameters and inhibitors.

You should have a first or second class honours degree or equivalent in microbiology or a related subject with a substantial bacteriology content and at least 2 years' relevant postgraduate research experience.

Appointment as Higher Scientific Officer £7785-£10,540 with starting salary according to qualifications and experience.

For further details and an application form (to be returned by 6 September 1985) write to Civil Service Commission, Alencon Link, Basingstoke, Hants RG21 1JB, or telephone Basingstoke (0256) 46855 (answering service operates outside office hours).

Please quote ref: SB/21/AJ.

The Civil Service is an equal opportunity employer

(7343)A

Scientific
Civil Service

Faculty Position available, tenure-track, in molecular genetics, with a special interest in membranes. Applicants should have research background in recombinant DNA technology (preferably also in somatic cell genetics) and working knowledge of membrane proteins. M.D. and/or Ph.D. in appropriate field required. Successful candidate will hold tenure-track appointment (Assistant or Associate Professor, depending upon qualifications) in Department of Physiology and Molecular Biophysics at Baylor College of Medicine. Joint appointments in relevant academic departments also possible. Laboratory space and basic equipment provided and initial laboratory support available. Successful candidate will devote majority of his/her effort to establishing and maintaining an independent research program related to the main interests of the Department. The Department is building a strong program in membrane biophysics with expertise in electrophysiology, membrane biochemistry, reconstitution, and modelling. **Send vita, a brief description of past and present research experience, and names of three or more references to**

**Dr. A. M. Brown, Incoming Chairman,
Physiology and Molecular Biophysics Department**



**Baylor College of Medicine,
One Baylor Plaza,
Houston, Texas 77030.**

An Equal Opportunity A/A Employer

(NW58)A

POSTDOCTORAL POSITIONS

available immediately to participate in ongoing studies involving the organization of dengue virus type 2 RNA genome using cDNA cloning, sequencing and expression of the viral genes in *E. coli*. Ph.D. degree in biochemistry or microbiology is required. Research experience or knowledge in molecular biology is desirable but not essential. Salary is \$16,000 — \$20,000 depending on experience. **Send curriculum vitae and names of three references to the following: Dr. Radha Padmanabhan, Associate Professor Department of Biochemistry, University of Kansas Medical Center, 39th and Rainbow Blvd. Kansas City, KS 66103. (NW119)A**

UNIVERSITY OF FLORIDA SENIOR LABORATORY RESEARCH TECHNICIAN in the Pathology Department

Experience in cell biology or immunology required. Work involves flow cytometry, cell culture and immunoassays. Independent research encouraged. Minimum qualifications are a bachelor's degree in Biology and two years of professional experience.

Base salary is \$18,061, negotiable, commensurate with experience.

Send complete résumé to Central Employment Center, 4th Floor Stadium, University of Florida, Gainesville, FL 32611 by 9/13/85. Requests must refer to position ID# 42213 in order to guarantee consideration.

Equal employment opportunity/affirmative action employer.

(NW120)A

The Queen's University of Belfast LECTURESHIPS

Department of Pure and Applied Chemistry

Applications are invited for two lectureships in Inorganic Chemistry, tenable from 1st October, 1985 or such other date as may be arranged. One of the lectureships is a 'New Blood' post for which candidates should preferably be under the age of 35 and not already hold a permanent University appointment in the United Kingdom. The successful candidates will probably have previous research experience in one of the following areas: main group chemistry, organometallic chemistry, bio-inorganic chemistry, inorganic solid state chemistry, although well qualified candidates with other fields of interest are encouraged to apply.

Salary range: £7,521 - £14,925 per annum with contributory pension rights under USS, initial placing depending on age and experience. One of the appointments will be made within the first four points on the scale. £7,521, £7,980, £8,451, £8,922 and will be subject to a period of probation of up to three years.

Further particulars may be obtained from the Personnel Officer, The Queen's University of Belfast, Northern Ireland, BT7 1NN. Closing date: 23rd September 1985

(7206)A

UNIVERSITY OF EDINBURGH Human Genetics Unit POST-DOCTORAL/ GRADUATE RESEARCH ASSOCIATE

A post-doctoral or graduate research associate is needed to establish, organise and manage a prototype DNA service laboratory for the East of Scotland. The objective will be to obtain newly developed DNA probes from other laboratories and to demonstrate their usefulness in prenatal diagnosis and carrier detection of human genetic diseases. Since the Human Genetics Unit is associated with the MRC Clinical and Population Cytogenetics Unit, there will be opportunity for original research in association with the latter Unit's molecular biology group.

Applicants should have organisational skills, a commitment to clinically useful work and the ability to communicate with the medical profession. Experience in standard DNA techniques is essential, as is knowledge of the use of linkage in diagnosis of genetic disorders

The post is funded by a grant from the Scottish Home and Health Department, running to April 1988 in the first instance.

Salary within the range £7,520 to £8,920 (1A) or £6,600 to £8,920 (1B) depending on age and experience.

Please apply to Dr D J H Brock, Human Genetics Unit, Western General Hospital, Edinburgh, EH4 2XU, enclosing a curriculum vitae and the names of two referees. Informal enquiries may be made to Dr Brock (Tel 031 332 7917).

Please quote Reference No 5273.
(7345)A

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF GEOLOGY TECHNICIAN GRADE 3

in the recently established Sedimentology Laboratory. The successful applicant will be responsible for operating a range of sedimentological research techniques, assisting in training students in these techniques and maintaining the laboratory. Experience of grain-size analysis, heavy mineral separation, cathodoluminescence microscopy, impregnation and staining techniques as applied to sediments and sedimentary rocks is required.

Candidates must possess HNC or equivalent as a minimum qualification but applications from graduates in Earth Sciences will also be welcomed. A minimum of eight years experience is also required.

Salary within the range £6,581 — £7,684 per annum (under review).

Application forms may be obtained from the Registrar, the University, PO Box 147, Liverpool, L69 3BX.

Quote Ref: PER/173/N

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY POSTDOCTORAL POSITION IN MOLECULAR PARASITOLOGY Department of Pure and Applied Biology POSTDOCTORAL SCIENTIST

required for a new development the immunology and molecular biology of filarial nematode parasites man. This position will involve combination of monoclonal antibodies and recombinant DNA technologies in the immunodiagnosis human filariasis, a major tropical disease. The successful applicant will join an existing research group at South Kensington site where the techniques are established. With familiarity with one of these fields would be advantageous, no previous experience of working with parasites would be necessary.

The project has been funded two years, renewable for a third, and the position is available immediately although appointment may be delayed for a suitable applicant. Salary will be on scale 1A, current £8,753 - £13,380 (under review) including London Allowance. Applicants should submit their cv and names of two referees by 6 September 1985 to Dr R M Maizels, Dept Pure and Applied Biology, Imperial College, London SW7 2BB, from whom further particulars may be obtained on 01-589-5111 ext 7485. (7349)A

UNIVERSITY OF MALAYA PETROLEUM GEOLOGIST

Applications are invited for a visit appointment of approximately year, beginning mid 1986. The appointment may be extended or renewed by mutual agreement.

Experience in petroleum industry is essential. Applications are especially invited from academicians who may be on sabbatical leave or who have retired from the industry. Salary will depend on the qualifications and experience of the candidate and will be within the range US\$15,000 to US\$30,000 annum. Air passage and accommodation will be provided.

The appointee will be responsible for planning and conducting undergraduate instruction in Petroleum Geology and related courses, and developing research co-operate with the petroleum industry.

Applications forms can be obtained from the Deputy Registrar (Establishment), University of Malaya, Kuala Lumpur 22-Malaysia. Applications should reach the Deputy Registrar before November 15, 1985. The Head, Department of Geology, University of Malaya, can be contacted for further

**THE NATURE
CONSERVANCY COUNCIL
HIGHER SCIENTIFIC
OFFICER**

2 year contract Higher Scientific Officer to work as an assistant on the National Countryside Monitoring Scheme

The National Countryside Monitoring Scheme (NCMS) aims to monitor habitat change throughout the British Isles using photogrammetric and computer based systems. The assistant will be part of a small team working on the NCMS project and his/her primary task will be the running of the digital mapping system which has been specifically designed for this project

The post will initially be based in Edinburgh, however, it is anticipated that the assistant will move with the project to the Peterborough office at a later date.

Qualifications and experience. Applicants should have a degree in a relevant subject and at least 2 years postgraduate experience. A good knowledge of computing and in particular digital mapping systems is required as is experience with aerial photographs. Some ecological background would be useful.

Salary depending on qualifications and experience: £7,788 rising to £10,541.

For an application form and further details of the post, please contact Miss B A Meredith, Personnel I, Nature Conservancy Council, Northminster House, Peterborough, PE1 1UA, to whom completed forms should be returned by 31 August 1985.

The Nature Conservancy Council is an equal opportunity employer. (7335)A

**MEDICAL RESEARCH
LABORATORIES,
THE GAMBIA
RESEARCH OFFICER IN
IMMUNOLOGY**

Applications are invited for a post of research officer in immunology at the MRC Laboratories in The Gambia. The successful candidate will be involved in studies in the immunology of human malaria and, in particular, in the development of human monoclonal antibodies to malaria antigens. He or she will work in close collaboration with scientists in laboratories in the UK. The successful candidate must have extensive previous experience in the characterisation and separation of antigens and antibodies; previous experience in parasitology is not essential. An initial contract of 2 years will be offered which may be renewed. Salary will be determined by qualifications and experience

Applications should be submitted to Miss K M Johns, MRC Head Office, 20 Park Crescent, London W1N 4AL from whom further details can be obtained. The closing date for applications is 30 August

**THE UNIVERSITY OF
MELBOURNE
RESEARCH FELLOW
GRADE 2
(EXTENDIBLE TENURE) IN
IMMUNOPARASITOLOGY**

in the
Department of Veterinary
Preclinical Sciences

Applications are invited from Immunologists/Immunoparasitologists interested in cellular immunity to cestode and trematode infections of sheep.

The research project is a collaborative study between the Sheep Biology Unit within the Department of Veterinary Preclinical Sciences and the Immunoparasitology Unit, Department of Veterinary Paraclinical Sciences. The Units are committed to ongoing projects on lymphocyte antigens, immunity to cestodes, cestode vaccines and lymphocyte recirculation using hybridoma and recombinant DNA techniques

The position is funded by the Wool Research Trust Fund and is available to 30th June 1986. The grant, however, has been awarded for a period of two years, with the possibility of further renewal. Commencement salary will be towards the base of the Research Fellow Grade 2 scale of \$A26,236 - \$34,467 per annum.

Further printed information regarding details of application procedure and conditions of appointment is available from the Appointments Officer (Academic), University of Melbourne, Parkville, Victoria 3052, Australia, or the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF

Applications, in duplicate, including names and addresses of at least three referees and quoting position number 260 A577, should be addressed to the Staff Officer, University of Melbourne, and close on 31 August 1985.

Equal opportunity in employment is the policy of the University of Melbourne. Selection is based on merit and all applications will be assessed regardless of sex, race, ethnicity or disability.

Applications from both men and women are invited for all vacancies. (W2102)A

**POSTDOCTORAL/
RESEARCH**

Associate position available to study RNA Processing in animal cells and viruses. Background either in Biochemistry, Microbiology or Molecular biology needed. Experience in recombinant DNA technology desirable. Salary negotiable.

Send resume with two names for references to Dr Mary Edmonds, Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260.

Equal Opportunity / Affirmative Action Employer

**CSIRO
AUSTRALIA
SENIOR PRINCIPAL RESEARCH SCIENTIST
\$A47,501 — \$A52,212
DIVISION OF ENTOMOLOGY
CANBERRA AUSTRALIA**

GENERAL: The Division carries out basic and applied biological research on insects and related arthropods so as to acquire knowledge relevant to the solution of problems of economic and social significance. The research involves the study of the identity, abundance, and distribution of insects; and of their behaviour, pathology and genetics. The Division is a major centre in Australia for such research and has a professional staff of 120.

The Division's research activities are grouped into a number of sections, each headed by a senior scientist whose role includes providing scientific leadership, control of resources and advice to the Chief of the Division on the research direction of the section, as well as actively initiating and maintaining personal research projects.

The Division has recently established a molecular biology program to investigate the relevance of recombinant DNA to basic and applied entomology. Current projects include studies on eukaryote chromosome organisation and behaviour, gene structure and function, gene transfer technology, the molecular basis of insect development, and molecular analysis of entomophilic nematodes and their bacteria. The Division is also interested in commencing research on the molecular analysis of insect behaviour and communication.

DUTIES: A vacancy exists for a senior scientist to provide research leadership to the molecular biology, physiology, biochemistry and physical sciences programs. The appointee will be familiar with modern techniques of molecular biology and will be expected to develop a personal research program in molecular biology, physiology or biochemistry of insects.

QUALIFICATIONS: A PhD degree or equivalent qualifications in an appropriate field. An excellent record of contribution to research knowledge in this field is required, together with the capacity for research leadership of a high order.

TENURE: Indefinite with Australian Government superannuation benefits available.

SALARY RANGE: Senior Principal Research Scientist, but for exceptionally eminent candidates, consideration may be given to appointment at a higher level.

APPLICATIONS: Stating relevant personal particulars, including details of qualifications and experience, the names of at least two professional referees and quoting reference No A2107, should be directed to:

The Chief
CSIRO Division of Entomology
GPO Box 1700
CANBERRA ACT 2601

by 4 weeks from date of issue

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER. (W2113)A

**CHAIRMAN
DEPARTMENT OF MICROBIOLOGY
UNIVERSITY OF ALABAMA AT BIRMINGHAM**

The University of Alabama at Birmingham invites applications and nominations for the position of professor and chairman of its Department of Microbiology. The candidate should have a strong commitment to academic excellence, and national recognition for scholarly accomplishments.

Please submit curriculum vitae and other pertinent information to:

Dr. J. D. Neill
Department of Physiology and Biophysics
University of Alabama at Birmingham University Station
Birmingham, Ala. 35294

An Equal Opportunity/Affirmative Action Employer

(NW1221A)

TWO POSTDOCTORAL FELLOWS/RESEARCH ASSOCIATES POSITIONS

available in CDNA and genetic cloning
DNA sequencing, recombinant
DNA protein interactions applied
to regulation of transcription and in
RNA translation.

Candidates should have a Ph.D. degree and experience in genetic engineering, proteins and nucleic acid biochemistry. Stipend from \$21,000, depending upon experience.

Application including curriculum vitae, one letter of reference and names of two additional references should be sent to:

**Ms. Elizabeth Beattie,
Department of Medicine,
New York Medical College,
Valhalla, NY 10595.**

(NW116)A

UNIVERSITY OF BRISTOL DEPARTMENT OF BIOCHEMISTRY AND UNIT OF MOLECULAR GENETICS COORDINATED PROGRAMME IN PROTEIN ENGINEERING

The SERC Biotechnology Directorate and several industrial sponsors have recently established an initiative to stimulate research in protein engineering. Bristol, is one of a small number of centres chosen for this coordinated programme.

Applications are invited for a MOLECULAR BIOLOGIST

of post-doctoral (Research Assistant 1A) status, preferably with some cloning and/or M13 sequencing experience.

Initially, the project will involve the cloning and sequence determination of phosphoglycerate kinase (PGK) genes isolated from thermophilic bacteria. Comparison of these sequences with the known sequence of yeast PGK will then form the basis of a multidisciplinary project (involving protein engineering, protein structure determination, computer graphics, etc.) aimed at establishing and exploiting the factors affecting protein thermal stability.

The position, which is available immediately, is initially tenable for three years with a starting salary in the range £7,520 — £8,920 p.a. according to age and experience.

Applications, including curriculum vitae and the names of two referees, should be sent as soon as possible to Dr L Hall at the Department of Biochemistry, University of Bristol, Medical School, Bristol, UK. (7312)A

THE UNIVERSITY OF SHEFFIELD DEPARTMENT OF PHYSICS SPACE PLASMA PHYSICS RESEARCH

We wish to recruit research staff at a postdoctoral level within the Space Physics Group, to be responsible for the scientific direction of investigations into plasma processes in the earth's magnetosphere and the solar wind.

One post is to study plasma motions and structures in the inner magnetosphere, both by observing natural VLF radio waves and by measuring Doppler shifts of a whistler mode signal from a northern hemisphere VLF transmitter, at Faraday station, Antarctica. A special receiver is currently under construction and will be deployed in early 1986.

The other post is concerned with the utilisation of data from the AMPTE satellites, to study wave-particle interactions in the outer magnetosphere and the solar wind. This will involve the data from the UKS wave experiment and other instruments on the UKS and IRM spacecraft.

Both posts will involve working with existing small teams and are available immediately for about two years in the first instance. Salary is on the RAI scale (£7,520 — £11,675, under review) according to age and experience. Letter of application and curriculum vitae should be sent to Professor G Turner FRS, Head of Department, Department of Physics, Sheffield S3 7RH (from whom further particulars are available) by 30 August 1985. Please (7312)A

TAYSIDE HEALTH BOARD DUNDEE GENERAL HOSPITALS NINEWELLS HOSPITAL & MEDICAL SCHOOL MOLECULAR BIOLOGIST

A Postdoctoral Scientist is required for a newly established Molecular Genetics Laboratory which will apply recombinant DNA techniques to the diagnosis of human disease. The post is funded initially for 3 years and work will be undertaken in the cytogenetics section of the University Department of Pathology and will be supported by adequate technical and laboratory facilities. The Laboratory is a participating member of a National Molecular Genetics Consortium aiming to provide a comprehensive service for Scotland. Applications should be experienced in the application of recombinant DNA methods. The appointment will be graded as Senior Cytogeneticist, the salary is £9,415 - £12,173.

Application forms and job descriptions from the Personnel Department, Ninewells Hospital. Tel: Dundee 60111, Ext 2578, quoting Reference Number GH/25/85. Closing date for completed applications is 20th August, 1985 (7352)A

SOUTH BIRMINGHAM HEALTH AUTHORITY

Birmingham Accident Hospital

Applications are invited for the position of

RESEARCH ASSISTANT

whole-time (Basic Grade Biochemist) to work in the Skin Culture Laboratory, Birmingham Accident Hospital. 1 Year contract in the first instance, commencing September 1985. Salary £6,559

Please send CV for further details and job description to Unit Administrator, Birmingham Accident Hospital, Birmingham. B15 1NA. (7355)A

ST MARY'S HOSPITAL MEDICAL SCHOOL (University of London)

Norfolk Place, London W2 1PG RESEARCH TECHNICIAN

required for the Department of Clinical Pharmacology for a period of two years. The research, which is funded by the British Heart Foundation, will primarily involve studies on the pulmonary responses to hypoxia and the effects of drug treatment.

Candidates with a training in biochemical pharmacology are encouraged to apply. Experience in adrenergic pharmacology and/or receptor binding techniques may be preferred.

Starting salary £5,035 plus £1,084 p.a. London Allowance.

Applications in the form of a cv and names of two referees should be sent to Assistant Secretary (Personnel) at the above address no later

YORK UNIVERSITY DEPARTMENT OF EARTH AND ATMOSPHERIC SCIENCE

DYNAMIC METEOROLOGIST

The Department of Earth and Atmospheric Science at York University invites applicants for positions of University Research Fellow in the area of dynamic meteorology. The Fellowships are research positions funded by the Natural Sciences and Engineering Research Council (NSERC) at the level of Assistant Professor tenable for up to five years. It is expected that the successful candidates will be prime candidates for tenure stream appointments in the department as they become available in the next few years.

Further information can be obtained by contacting Prof. John C. McConnell, Chairman, Department of Earth & Atmospheric Science, York University, 4700 Keele Street, Downsview, Ontario M3J 1P3.

These positions are tenable only by citizens or permanent residents of Canada at the time of application. In order to meet the NSERC deadline of 1st November, for fellowships to be awarded in 1986, York will require formal application by 30th September 1985. (NW49)A

Applications are invited for a POSTDOCTORAL RESEARCH ASSISTANT

interested in joining a group working on gene regulation in African Trypanosomes. The candidate should have experience in the techniques of molecular genetics. Salary according to BAT III/IIa (DM 42,700, — DM 46,600, — pa).

Prof Dr P Overath, Max-Planck-Institut für Biologie, Corrensstrasse 38, 74 Tübingen, West Germany. (W2089)A

UNIVERSITY OF ST ANDREWS

DEPARTMENT OF BIOCHEMISTRY AND MICROBIOLOGY

Applications are invited from medical or science graduates for a Demonstratorship in the Department of Biochemistry and Microbiology. The Demonstrator will assist in the teaching of medical and science classes and opportunities for advanced study will be available.

Salary £8,450 per annum, plus superannuation. The appointment is for the period 1st October 1985 to 31st May 1986. The appointment may be renewable for the corresponding period in the following academic year.

Further particulars may be obtained from the Secretary, Department of Biochemistry and Microbiology. Applications (two copies preferably in typescript) with the names of two referees should be submitted, before 25 August 1985, to the Establishments Office, University of St Andrews, College Gate, St Andrews, Fife, KY16 9AJ. (7312)A

UNIVERSITY OF LONDON

The Cardiothoracic Institute
New Blood Appointment
in the Department
of Thoracic Medicine

Applications are invited from candidates intending to pursue a career in Academic Medicine or Research for a 'New Blood' appointment in the Department of Thoracic Medicine. The successful candidate is likely to be a clinical or non-clinical scientist with some experience in immunological, cell biological or microbiological research methods. He or she will be expected to contribute new ideas in this area of research and to carry them through experimentally within the current programme of research into cellular, biochemical and host defence aspects of pulmonary disease currently conducted in the Department of Medicine. It is anticipated that appropriate Honorary Status would be granted to a clinical appointee by Brompton Hospital.

Candidates should not hold a permanent University appointment in the UK nor exceed the age of 35 (non-clinical) nor 38 (clinical) years. Appointment will be on the Lecturer or Senior Lecturer Scale. A London Allowance is payable.

Applicants should apply by letter, enclosing 8 copies of their curriculum vitae, a statement of not more than 500 words on why they are applying for the post and the names and addresses of 3 referees to: The Secretary, Cardiothoracic Institute, Fulham Road, London SW3 6HP from whom further particulars can be obtained.

Closing date for applications: 13 September 1985.

(7334)A

UNIVERSITY OF OXFORD

DEPARTMENT OF
HUMAN ANATOMY

GRADUATE RESEARCH
ASSISTANT (Grade 1B)

A position is available in the department to work with Dr CD Stern on a project funded by the Medical Research Council, to study the roles played by cell lineage and by cell interactions in segmentation in vertebrate embryos. Applicants should have obtained, or should expect to obtain, a First Class or Upper Second Class Honours degree in Biology, Zoology, Pre-clinical Medicine or related subjects. The position will be available in October 1985 or as soon as possible thereafter, it will be tenable for three years and the salary will be according to the 1B scale: £6,600 — £7,520 per annum plus a conference allowance. The successful applicant may have the opportunity to register for a higher degree.

Applications in writing, including curriculum vitae and the names and addresses of two referees should be sent to: Dr CD Stern, Department of Human Anatomy, South Parks Road, Oxford OX1 3PS.

THE ROWETT
RESEARCH INSTITUTE

Bucksburn, Aberdeen,
AB2 9SB

2 Year Post-Doctoral
Appointment Funded by
Scottish Home & Health
Department
Biochemist/ Physiologist/
Pharmacologist
HSO/SSO Grade

A well qualified research scientist with a knowledge of lipid metabolism, arachidonic acid metabolism and cardiovascular physiology/ pharmacology is required to work under the supervision of Dr K W J Wahle in the Biochemistry Division.

The project relates to the effect of isomeric monoenoic fatty acids on PUFA metabolism and eicosanoid synthesis in cardiovascular tissues/ membranes.

Qualifications: First or upper second class honours degree and a PhD in a relevant subject. At least 4 years post-graduate experience is required for SSO grading.

Salary Scales: HSO £7,788 to £10,541 pa, SSO £9,772 to £12,653 pa with placing according to experience.

The position is Superannuable and the Institute is an Equal Opportunities Employer.

Applications in writing together with full CV and the names/addresses of 2 referees should be sent to the Secretary of the Institute from whom further particulars are available. Tel No 0224-712751 Ext 338.

Closing date: 29th August 1985.
(7327)A

UNIVERSITY OF
EDINBURGH

DEPARTMENT OF RESPIRATORY
MEDICINE

Applications are invited for the post of

RESEARCH ASSOCIATE

funded for 3 years by the Wellcome Trust to work on aspects of the control of breathing by hypoxia in conscious humans including 1) physiological mechanisms of the potentiation by exercise, 2) changes with age, and 3) the role of the response in the development of chronic bronchitis and emphysema. Applicants should hold a good Honours degree in a physiological science with at least 3 years relevant postgraduate experience and be under the age of 32 years. Appointment will be on the 1B scale (£7,980 pa).

Further information may be obtained from Professor D C Flenley, Department of Respiratory Medicine, City Hospital, Greenbank Drive, Edinburgh EH10 5SB to whom applications including names and addresses of two referees should be sent by 19th August.

Please quote reference No 5272.

CSIRO

AUSTRALIA

RESEARCH FELLOW

\$A26,001 — \$A32,083

DIVISION OF APPLIED
ORGANIC CHEMISTRY
FISHERMAN'S BEND VIC

GENERAL: The Division has a staff of approximately 100, including about 50 professional scientists. Investigations are carried out in programs of biological organic chemistry, industrial chemistry and polymer science. The Biosynthetic Chemistry group is part of the Biological Organic Chemistry Program, which carries out work in the synthesis of biologically active compounds for development and manufacture in Australia for the world market.

DUTIES: As a member of the Biosynthetic Chemistry group the appointee will collaborate in research on the synthesis and development of agrochemicals and pharmaceutical agents. The research involves multidisciplinary work in synthetic chemistry in several fields of biology, physical chemistry and computer design of the compounds.

QUALIFICATIONS: A PhD degree or equivalent qualifications in the field of organic synthetic methods and the operation and interpretation of data obtained using organic chemistry instrumentation e.g. NMR, IR, Mass Spectrometry, GLC, HPLC. Experience in the synthesis of biologically active compounds would be an advantage.

TENURE: A term of three years with Australian Government superannuation benefits available.

APPLICATIONS: Stating relevant personal particulars, including details of qualifications and experience, the names of at least two professional referees and quoting reference No A1051, should be directed to:

The Chief
CSIRO Division of Applied Organic Chemistry
GPO Box 4331
MELBOURNE VIC 3001

by 4 weeks from date of issue

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER (W2112)A

UNIVERSITY OF EDINBURGH

DEPARTMENT OF VETERINARY PATHOLOGY

WELLCOME TRUST LECTURESHIP IN MOLECULAR BIOLOGY

Applications are invited for a Wellcome Trust Lectureship in Molecular Biology in the Department of Veterinary Pathology, University of Edinburgh. The Department has a major interest and excellent facilities for basic research in immunology and the molecular aspects of pathology. The person appointed will join an active research group working on the immunology of persistent viral infection and will be expected to work on the molecular biology of pestiviruses (Border Disease virus of sheep, virus of Bovine Viral Diarrhoea — Mucosal Diseases of cattle) and investigate the molecular biology of the interactions between these viruses and the cells of immune systems in sheep. Molecular biology techniques have been introduced to the Department, and the successful applicant will be expected to further develop Molecular Biology in relation to the Department's research interests in immunology and its teaching programmes. Applicants must have research experience and a training in recombinant DNA technology and an interest in immunology.

The salary will be on the lecturers' scale (£7,190—£14,125). Starting salary depending on age, qualification and experience. The Lectureship is for three years in the first instance with renewal for a further two years on satisfactory review. Candidates should not be older than 35 years of age. Further particulars can be obtained from Professor I. McConnell, Department of Veterinary Pathology, University of Edinburgh, Summerhall, Edinburgh EH9 1QH (Tel: 031 667 1011 ext. 5322 or 5343). Candidates will be asked to submit a 500 word summary of the proposed research and should request the further particulars.

Applications (6 copies) giving the names of 3 referees should be sent to:

The Personnel Office, University of Edinburgh, 63 South Bridge, Edinburgh EH1 1LS.

Closing date for applications is 30th August, 1985, with appointment as soon as possible after that date. Applications from overseas should lodge their interest by this date with applications to follow.

Please quote reference No 5266.

(7190)A

ASSISTANT PROFESSOR

Department of Pathology is seeking a Hematopathologist for a Junior Faculty tenure accruing position. Candidate must have a M.D. degree and must have experience in diagnostic hematopathology. A strong interest in pursuing research activities in the areas of tumor biology, cellular immunology or molecular biology. It is expected that such an individual would share diagnostic responsibilities including interpretation of bone marrow and lymphoid tissue biopsies and immunophenotyping of normal and neoplastic leukocytes using modern flow cytometric techniques and tissue section labelling. The individual will also be involved in the training of pathology residents and graduate students. Recruiting deadline is October 1, 1985 with an anticipated start date of November 1, 1985.

Applications should be sent to: Paul C. Braylan, M.D. Professor, Department of Pathology, Box J-275, College of Medicine, University of Florida, Gainesville, Florida, 32610.

Equal Opportunity Employment/Affirmative Action Employer.
(NW129)A

UNIVERSITY OF CAMBRIDGE

Department of
Clinical Biochemistry

RESEARCH ASSISTANT

position available for 15 months starting asap. Would suit new graduate. Project involves developing the use of a series of monoclonal antibodies to immunoassay alkaline phosphatase isoenzymes. Send cv and names of 2 referees to Dr J P Luzio, Dept of Clinical Biochemistry, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QR.

(7316)A

MAX-PLANCK-INSTITUT FUER MEDIZINISCHE FORSCHUNG

DEPARTMENT OF
MOLECULAR BIOLOGY
POSTDOCTORAL FELLOW
IN MOLECULAR BIOLOGY

The position is available in a research group involved in a study of structure and regulation of human muscle genes and of the differentiation of muscle cells. The project includes cloning and sequencing of muscle genes with special emphasis on myosin heavy chain. The project will be extended into a biological analysis of cultured normal and dystrophic human myoblasts. Experience in molecular biology methods is essential. Expertise in cell culture techniques is desirable.

The appointment is supported by the Deutsche Forschungsgemeinschaft and will be for two years starting as soon as possible. The net salary is about DM 2,500.

Applications together with CV, research experience and interests, list of publications and the names of two referees should be sent to the administration of the Max-Planck-Institut fuer Medizinische Forschung, Jahnstr, 29 6900 Heidelberg, FRG, before 30th September, 1985.

(W2105)A

UNIVERSITY OF DUNDEE

DEPARTMENT OF PATHOLOGY
POST DOCTORAL
RESEARCH ASSISTANT

Applications are invited from appropriately qualified scientists to work on microcirculatory changes induced by lymphokines in a project funded by SHHD for three years. Postgraduate experience in bioassay of lymphokines, microcirculatory research or protein fractionation will be an advantage. The appointment will be on the Research & Analogous Staff Grade 1A, salary £7,520 — £12,150 (under review).

Informal enquiries may be made to Professor Beck (telephone 0382 60111 extension 2169).

Applications, including a curriculum vitae and the names of two referees, should be sent to The Personnel Department, The University, Dundee by 29th August 1985. Please quote reference EST/83/85J.

(7356)A

CHARING CROSS AND WESTMINSTER MEDICAL SCHOOL (University of London) RESEARCH ASSISTANT

Recent graduate in a Biological Science required for a research project in the Department of Histopathology and in the Kennedy Institute of Rheumatology, on the transfer of lysosomal enzymes between cultured cells. Experience in electron-microscopy and cytochemistry an advantage but training in relevant techniques will be given. Appointment for two years on salary scale £6,600 — £7,050 pa plus £1,233 pa London Allowance.

Applications, with full curriculum vitae and the names and addresses of two referees to The Secretary, Charing Cross and Westminster Medical School, The Reynolds Building, St Dunstan's Road, London, W6 8RP, to be submitted within three weeks of the appearance of this advertisement.

(7322)A

ROYAL POSTGRADUATE MEDICAL SCHOOL (Hammersmith Hospital)

DEPARTMENT OF MEDICINE
(Division of Gastroenterology)

POSTDOCTORAL
PHYSIOLOGIST or
PHARMACOLOGIST

To study roles of regulatory peptides in gastrointestinal secretion and motility on a 2 year project funded by the Wellcome Trust. Basic in vitro techniques required. The work is in an expanding field and aims to improve the treatment of human disease.

The appointment is to begin on or after 1 October 1985. Salary will be on scale £7,980 to £8,450 plus £1,233 London Allowance a year (under review).

Application forms may be obtained from the Personnel Officer, Royal Postgraduate Medical School, 150 Ducane Rd, London W12 0HS quoting reference 2/496.

Closing Date: 29 August, 1985.
(7324)A

UNIVERSITY OF BIRMINGHAM

Faculty of Medicine and
Dentistry

DEPARTMENT OF PHYSIOLOGY

Applications invited to work on an MRC funded research project on the mechanisms of regulation on contraction in smooth muscle under the supervision of Professor S V Perry. Candidates should have a degree in Biochemistry.

Post for three years salary Research Associate 1B — Research Fellow 1A £6,600 — £8,920 £7,520 — £12,150 with superannuation.

Further particulars from Assistant Registrar, Medical School, University of Birmingham B15 2TJ, to whom applications (3 copies) should be sent by 31 August 1985. Quote Ref RA/Phys/SVP.

(7353)A

UNIVERSITY COLLEGE CARDIFF

DEPARTMENT OF BIOCHEMISTRY
RESEARCH ASSISTANT IN
BIOCHEMISTRY

Applications are invited for the above post to work on a project funded by SERC to develop biocatalytic methods for the production of optically active compounds. Applicants should hold or expect to gain a degree in Biochemistry or Microbiology. The appointment is for 3 years starting 1 October 1985 at salary of £6,600 per annum.

Applications (2 copies), together with the names and addresses of two referees should be sent as soon as possible to the Establishment Officer, University College Cardiff, PO Box 78, Cardiff CF1 1XL, from whom further particulars are available. Closing date 6th September, 1985.

1985 D.F. 2000

(7309)A

ROYAL POSTGRADUATE MEDICAL SCHOOL

(University of London)

Cancer Research Campaign Cell
Proliferation Unit

DEPARTMENT OF
HISTOPATHOLOGY

RESEARCH ASSISTANT

required to assist with studies on growth and differentiation of human colorectal adenocarcinoma cells in vitro. The successful applicant will work with Dr S C Kirkland and will be funded by a CRC program grant.

Previous experience in tissue culture techniques is highly desirable.

Salary on range £5,316 - £7,682 plus £1084 London Allowance a year.

Application forms from the Personnel Office, Royal Postgraduate Medical School, 150 Ducane Road, London W12 0HS quoting ref: 6/202/N.

Closing date: 22 August 1985.
(7333)A

THE MIDDLESEX HOSPITAL MEDICAL SCHOOL

(University of London)

London W1P 7PN

COURTAULD INSTITUTE
OF BIOCHEMISTRY
RESEARCH ASSISTANT/
TECHNICIAN

to investigate factors affecting metabolism of carcinostatic drugs. The project will utilize changes in microbial genetic markers as a measure of metabolism. The post, funded for two years, would suit a recently qualified graduate in microbiology or genetic toxicology. Starting salary up to £7,833 inclusive of London Allowance.

Applications, with CV, should be sent to Dr S Neale, Courtauld Institute of Biochemistry at the above address.

(7360)A

BAKER MEDICAL RESEARCH INSTITUTE Melbourne, Australia PROTEIN BIOCHEMIST

Applications are invited for a Senior Research Officer in the Lipoprotein Biochemistry Laboratory at the Institute. The position requires a person with Postdoctoral experience in protein chemistry, preferably related to lipoproteins although this is not essential. Our research explores biochemical relationships between lipoprotein metabolism and atherosclerosis, requiring protein isolation, use of immunochemical techniques, cell biology, etc.

Candidates should submit a curriculum vitae containing research experience, publications and names, addresses and telephone numbers of three referees to Dr N Fidge, Baker Medical Research Institute, Commercial Road, Prahran, Victoria, Australia 3181.

(W2006)A

UNIVERSITY OF BRISTOL

POSTDOCTORAL
RESEARCH ASSOCIATE

STABILITY OF
NON-AQUEOUS
DISPERSIONS

Investigations into the role of electrostatic interactions in controlling the stability/flocculation behaviour of colloidal dispersions in non-aqueous media require the accurate measurement of surface charge. To this end, it is intended to develop either a laser-Doppler electrophoretic technique for use with non-aqueous dispersions.

The project will be for two years starting on or after October 1st 1985, and will be carried out in the Physical Chemistry Department at Bristol University with Dr B. Vincent. The starting salary will be at a suitable point on the University IA scale range of £7,520 to £12,150, depending on age and experience.

Applicants, with a suitable background in physical chemistry or physics, should write to Dr B. Vincent, Dept. of Physical Chemistry, University of Bristol, Bristol BS8 1TS, as soon as possible. A curriculum vitae, together with the names and addresses of two referees, should be included. (7319)A

UNIVERSITY OF
LEICESTER

DEPARTMENT OF
MICROBIOLOGY

POST DOCTORAL
RESEARCH ASSOCIATE
RESEARCH TECHNICIAN
(Grade III)

Molecular Biology of Bacterial
Virulence

Applications are invited for these two positions funded by the Medical Research Council, and tenable for up to three years from 1st October 1985. Salary according to age, experience and qualifications.

The work will involve the detailed molecular analysis of surface structures of *Escherichia coli* involved in mediating adhesion to human cells. This is a collaborative project with Prof K Jann, Max Planck Institute for Immunobiology, Freiburg, West Germany and will involve exploitation of monoclonal antibodies and techniques of gene cloning and analysis. Experience in recombinant DNA, immunology and/or protein biochemistry would therefore be an advantage.

Applications, in the form of a curriculum vitae with the names of two referees, should be sent to Dr G J Boulnois, Department of Microbiology, Medical Sciences, University of Leicester, University Road, Leicester, LE1 7RH, UK (Tel 0533-51234, Ext 262). (7341)A

Please mention

nature

when replying to
these advertisements

UNIVERSITY OF
CAMBRIDGE

DEPARTMENT OF PARASITOLOGY
UNIVERSITY LECTURER

Applications are invited for a post of University Lecturer in the Department of Parasitology, appointment to commence on 1 January 1986 or as soon thereafter as possible. (In 1987 the Department of Parasitology will unite with the Department of Pathology to form a single department.)

Candidates should hold a PhD degree or equivalent and have several years post doctoral experience in parasitology and in the application of molecular, biochemical or immunological techniques to the investigation of the developmental biology of parasites and of host/parasite relationships. The successful candidate will be expected to teach natural science, medical and veterinary students at undergraduate and postgraduate levels.

Stipend: £9,875 by eleven annual increments of £15,085 (under review).

Further information about the duties and conditions of appointment may be obtained from the Secretary of the Appointments Committee for the Faculty of Biology 'A', 19 Trumpington Street, Cambridge, CB2 1QA, to whom applications (10 copies) including curriculum vitae, list of publications and the names of not more than three referees, should be sent so as to reach him not later than 1 October 1985. (7314)A

LECTURER IN
PHYSIOLOGY

University of Cape Town/
Cape Provincial Administration

Applications are invited for the above post for appointment as soon as possible. Preference will be given to applicants with interests in neurophysiology, renal physiology or mineral metabolism.

The post is full-time and under the terms of the joint agreement between the University and the Cape Provincial Administration.

Salary range R20 482 - R34 070 per annum with an annual bonus and attractive staff benefits.

Applicants should submit a full curriculum vitae stating qualifications, teaching and research experience, interests and publications, present salary and the names and addresses of three referees not later than 9 September 1985 to the Registrar, (Attention: Appointments Office Ref: AC/5714) University of Cape Town, Private Bag, Rondebosch, 7700, South Africa. Further information may be obtained from the Registrar or The Secretary, SA University Office, Chichester House, 278, High Holborn, London WC1V 7HE.

The University's policy is not to discriminate on the grounds of sex, race or religion. Further information on the implementation of this policy is obtainable on request. (W2092)A

M.R.C. CLINICAL RESEARCH CENTRE
(NORTHWICK PARK HOSPITAL)
WATFORD ROAD, HARROW
MIDDLESEX HA1 3UJ



NON-CLINICAL SCIENTIST

Applications are invited from post-doctoral microbiologists for a 3-year short-term appointment to study the host-parasite relationships between mammalian cells and bacteria, viruses and mycoplasmas. The research programme will be concerned with identification of glycoprotein or glycolipid receptors for infective agents on the host-cell membranes.

The successful candidate will join the Applied Immunochemistry Research Group headed by Dr Ten Feizi from whom further information can be obtained (Tel 01-864 5311 ext 2352).

Salary will be within the range £10,153 — £16,158 (inclusive of London Weighting) depending on experience. Application forms are obtainable from Miss B A Shaw, Personnel Section, quoting reference 108/1/3905.

Closing date for applications 15th August 1985

(7311)A

UNIVERSITY OF
NOTTINGHAM

Department of Zoology

POSTDOCTORAL
RESEARCH ASSISTANT

A one-year post is available from 1st October 1985 to work on a SERC funded project looking into risk-sensitive and time-constrained foraging in shrews. Applicants should have a working knowledge of optimal foraging theory and experience with laboratory behavioural studies. The appointment will be within the first two points of the post-doctoral scale.

Applications to be sent as soon as possible with curriculum vitae to Dr C J Barnard, Department of Zoology, University of Nottingham, University Park, Nottingham, NG7 2RD. (7332)A

UNIVERSITY OF
BIRMINGHAM

Faculty of Medicine and
Dentistry

DEPARTMENTS OF IMMUNOLOGY
AND PHYSIOLOGY
RESEARCH FELLOW/
ASSOCIATE

Applications invited for the above post to investigate the use of monoclonal antibodies to identify and characterise cardiac specific proteins.

Post available for up to two years funded BHF grant to Dr G K Dhoot and Professor S V Perry starting October 1985. Salary on scale £6,600 — £8,920 or £7,520 — £12,150 with superannuation.

Candidates should have research experience in protein chemistry and/or immunochemical techniques.

Further particulars Assistant Registrar, Medical School, University of Birmingham, B15 2TT, to whom applications (3 copies) should be sent by 3 September 1985. Quote Ref RA/IMP/GD. (7354)A

UNIVERSITY OF
CAMBRIDGE

DEPARTMENT OF BIOCHEMISTRY
MOLECULAR BIOLOGIST

to work on the control of germination of castor beans. The work involves the use of probes for the mRNA of purified enzymes from glyoxysomes construction of genomic clones, DNA sequencing and the application of plant growth factors. This is funded for 11 months from 1st October 1985.

Salary according to age and experience £7,520 — £9,860 (under review).

Applications should be sent as soon as possible to Professor D H Northcote, FRS, Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge, CB2 1QW (7323)A

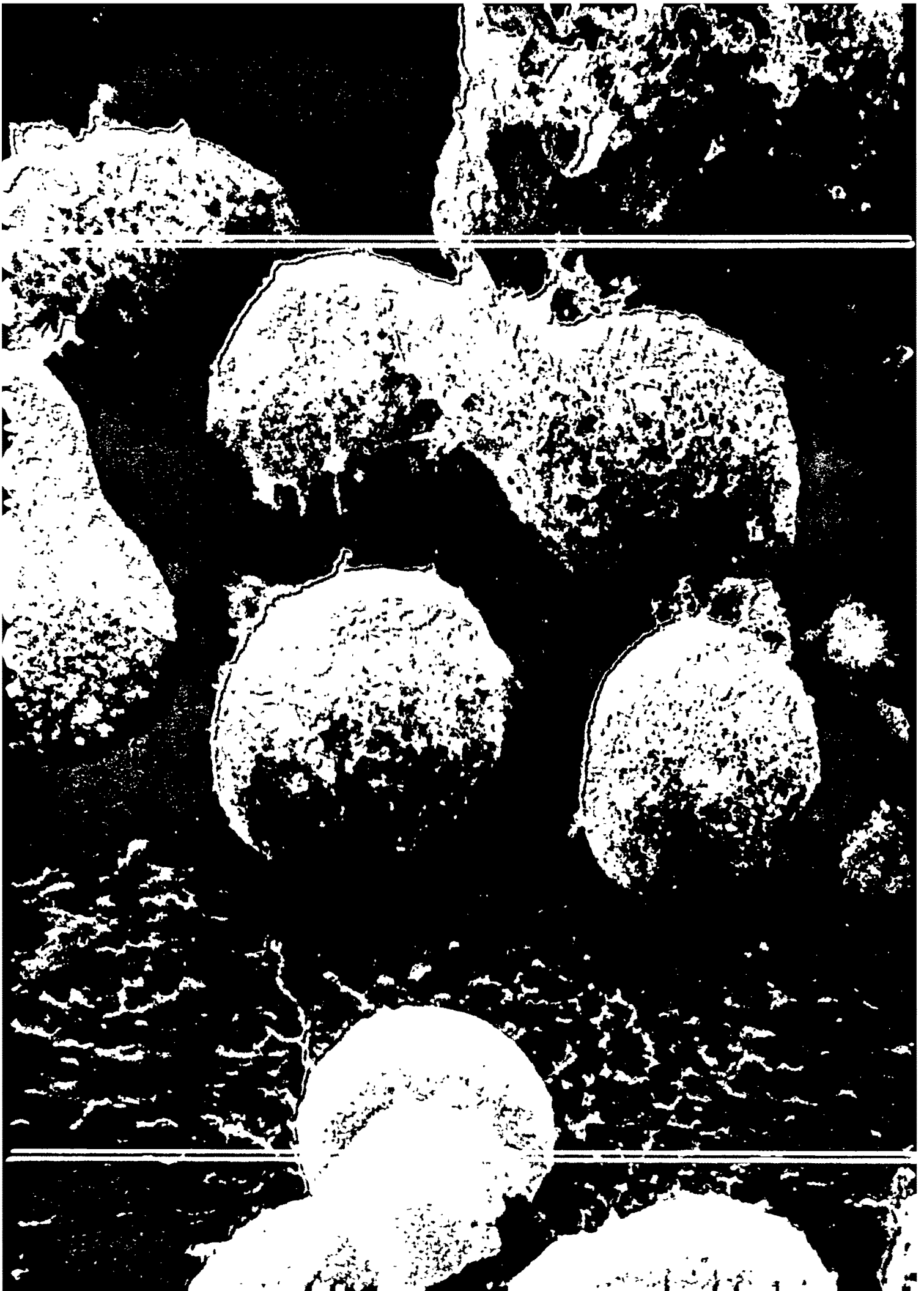
UNIVERSITY OF BRISTOL

DEPARTMENT OF
MICROBIOLOGY AND UNIT OF
MOLECULAR GENETICS

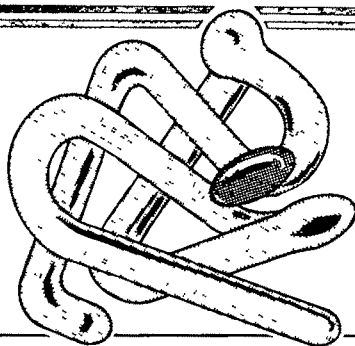
PREDOCTORAL
RESEARCH ASSISTANT

Applications are invited, as soon as possible, for the above post, which is supported by the Medical Research Council for 3 years. The work will examine structure-function relationships in *E coli* membrane proteins concerned with tetracycline transport and efflux. Applicants should have an honours degree in a biological science preferably with some training in molecular genetics, bacterial resistance plasmids or bacterial cell membranes.

The starting salary will be £6,600 rising to £7,520 per annum in the third year (Research Range 1B; salaries under review), and the successful candidate may be able to register for a higher degree. Further details can be obtained from Dr Ian Chopra, Department of Microbiology, The Medical School, University Walk, Bristol, BS8 1TD, to whom applications, including curriculum vitae and the names of two referees should be sent. (7337)A

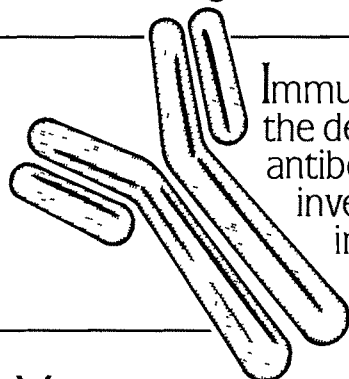
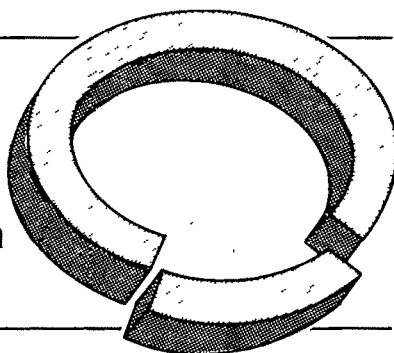


Have you got the right chemistry?



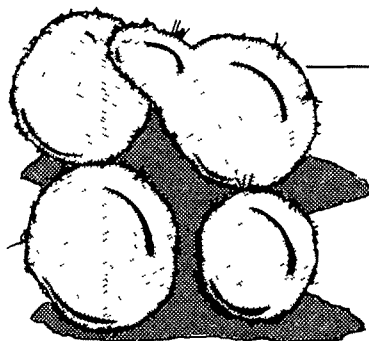
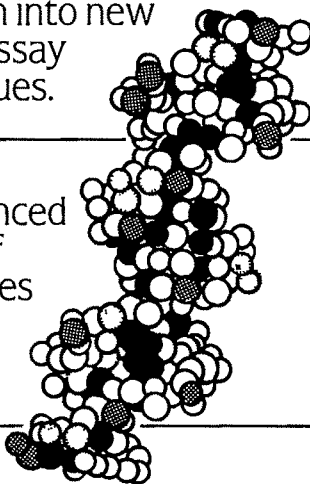
Protein chemists experienced in the purification and physico-chemical analysis of proteins and antigens.

Recombinant DNA scientists experienced in cloning DNA fragments into plasmid vectors and nucleic acid isolation from mammalian genes.



Immunologists experienced in the development of enzyme-antibody conjugates and investigation into new immunoassay techniques.

Molecular geneticists experienced in gene identification, design of synthetic oligonucleotide probes and hybridisation techniques.



Cell biologists experienced in cell physiology, tissue culture and hybridoma - monoclonal antibody technology.

ICI is currently going through a period of major diversification and expansion into the medical diagnostics field with the establishment of the new ICI Diagnostics Group. Backed by substantial scientific and financial resources, the new group is pursuing a broad strategy embracing in-house research, product licensing and collaborative projects designed to make the company a major force in diagnostics.

Shown here is the range of scientific personnel we seek to join a new and dynamic research team involved in the development of nucleic acid - and immunoassay - based diagnostics for medical applications. Emphasis will be strongly based on the design of new diagnostics and the use of non-isotopic detection technology.

We are interested in applicants holding a good first degree or suitable equivalent qualifications, and those holding a Ph.D with, preferably, postdoctoral experience. Candidates should have a broad interest in the diagnostics field together with a flexible and adaptable approach to research areas geared to the market place. This will require a rare combination of both research and practical interest in the development of non-isotopic nucleic acid and immunoassay systems.

In return, we offer excellent technical facilities in attractive 'Greenfield' surroundings, opportunities for rapid career progression as the group expands, highly competitive salaries and conditions and a generous relocation package.

We intend to hold a series of regional meetings during September when selected applicants will be invited to find out more about our move into medical diagnostics.

Those who wish to take advantage of this exciting opportunity should send details of their career to date to Dr Trevor Twose, R & D Manager.

 **Diagnostics Group**

Stuart House, 50 Alderley Road,
Wilmslow, Cheshire SK9 1RE, UK.
Telephone: 0625 522022

OXFORD POLYTECHNIC
Department of Biology
RESEARCH ASSISTANT
Physiological Psychology
(Salary on scale A)

£5,424-£6,153 limited tenure)
Applications are invited for the post of Research Assistant tenable for 1 year from October 1st to complete work on an MRC funded project on Brain and Behavioural Correlates of Early Undernutrition under the direction of Dr T C Jordan. The post offers considerable opportunity for varied work, including behavioural and physiological assessment of hippocampal function. Experience with electrophysiological recording and the use of microprocessors would be an advantage.
Applications, enclosing a curriculum vitae and names of two referees by Sept. 1st to The Secretary, Biology Department, Oxford Polytechnic, Headington, Oxford OX3 0BP.
An equal opportunities employer (7372)A

UNIVERSITY COLLEGE LONDON

Department of Anatomy and Embryology
RESEARCH ASSISTANT (1A)

This postdoctoral position is available for one year in the first instance, but with the possibility of extension up to two years on an MRC grant concerned with electrophysiological studies of the actions and release of neuropeptides at synapses in intramural autonomic ganglia. The technical and intellectual environment for research is attractive. Salary in the range £9,390 - £10,330 + £1,233 LA. Applications, together with full cv and names and addresses of two referees to Professor G Burnstock, Department of Anatomy & Embryology, University College London, Gower Street, London WC1E 6BT. (7346)A

KING'S COLLEGE LONDON

DEPARTMENT OF BIOPHYSICS
Applications are invited for a
POST-DOCTORAL RESEARCH ASSOCIATE

to work with Dr Roger Patient on the role of DNA supercoiling in establishing active chromatin structure and transcriptional activation, using tissue culture transfection and *Xenopus* oocyte injection

The post will be tenable for up to three years from January 1986. Salary in the range £7,520 - £12,150 pa plus £1,233 London weighting.

Further details may be obtained from Mr D M Drummie, MRC Cell Biophysics Unit, 26-29 Drury Lane, London WC2B 5RL, whom applications should reach by 29 August 1985, or by telephoning Dr Patient on 01 836 8851 (7312)A

KING'S COLLEGE LONDON (KQC)

University of London
Department of Biochemistry
POSTDOCTORAL RESEARCH ASSISTANT

A Postdoctoral Research Assistant is required to work with Dr J M Wrigglesworth on a SERC funded project to investigate the redox-linked Bohr properties of cytochrome c oxidase. The post will be available for two years from 1st October 1985 with a starting salary of £8,753 per annum inclusive.

Applications (with curriculum vitae and names and addresses of two referees) should be sent to Dr J M Wrigglesworth, Department of Biochemistry, King's College London (University of London), Campden Hill Road, Kensington, London W8 7AH.

Closing date: 30th August 1985. (7336)A

THE UNITED MEDICAL AND DENTAL SCHOOLS
Guy's Campus
POSTDOCTORAL RESEARCH ASSISTANT

required to join a team working on regeneration in the nervous system, with particular emphasis on the central nervous system. Applicants should have experience in neurohistological and immunocytochemical techniques; also electron microscopy and/or stereological techniques would be an advantage.

Further information from Dr S Standring, Department of Anatomy 01-407 7600 Ext 3349.

Applications, with full CV and names of two referees, to the Staffing Officer, Guy's Hospital Medical School, London SE1 9RT, quoting Ref: G/A/89, by 29 August 1985. (7366)A

UNIVERSITY OF OXFORD

DEPARTMENT OF AGRICULTURAL SCIENCE
RESEARCH ASSISTANT

Applications are invited from persons with a PhD or comparable research experience to develop a predictive model for the convective transport of solutes, especially nitrate, through soil. In addition to soil scientists, applied mathematicians, physicists and engineers interested in flow through porous media are invited to apply. The post, on Research Assistant scale 1A, will be funded by the AFRC for 3 years.

Applications, with curriculum vitae and the names of two referees, should be sent as soon as possible to Dr R E White, Department of Agricultural Science, Parks Road, Oxford, OX1 3PF, from whom further particulars can be obtained (7364)A

UNIVERSITY OF NEWCASTLE UPON TYNE
DEPARTMENT OF NEUROLOGY
POSTGRADUATE RESEARCH STUDENTSHIP

Applications are invited for a research studentship tenable in the Muscular Dystrophy Group Research Laboratories, Newcastle General Hospital. The successful applicant will read for the degree of PhD. Two topics are available: (i) investigation of genetic or acquired defects of the mitochondrial respiratory chain in human muscle disease using biomedical, cytochemical and immunological techniques; (ii) the use of cytochemical techniques to study the molecular differentiation of the muscle cell surface during development and regeneration of the neuromuscular junction.

Candidates should be appropriately qualified and possess at least a 2.1 Honours degree. Applications should be made as soon as possible in the form of a curriculum vitae, and candidates should include the names of two referees. The project for which the application is being made should also be identified. All correspondence should be sent to Professor J B Harris, Muscular Dystrophy Group Research Laboratories, Newcastle General Hospital, Westgate Road, Newcastle upon Tyne, NE4 6BE, from whom further information may be obtained. (7365)A

UNIVERSITY OF KEELE

DEPARTMENT OF PHYSICS
and SERC Daresbury
Laboratory

POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for a post funded by an SERC project grant Dr R J Greenall, Professor W Full and Dr G P Diakun for the investigation of the cation environment fibres of the polynucleotide poly d(A-T) poly d(A-T) using EXAFS and X-ray fibre diffraction. The post is available for three years from 1 October 1985 and the appointment will be made on Research Scale 1; salary range £7,520 - £12,150 p annum.

Application forms and further particulars are available from the Registrar, University of Keele, Keele, Staffs, ST5 5BG to whom completed forms should be returned no later than Wednesday 28 August 1985. (7368)A

ASSOCIATESHIPS

THE UNIVERSITY OF LANCASTER
Department of Physics
CHARACTERISATION OF DEFECTS IN SOLIDS USING PHONONS

Applications are invited for a 3-year, SERC financed, research associateship to work with Drs R A Collins and J K Wigmore on the generation of defects in semiconductors and insulators using ion beam techniques at AERE Harwell, and their characterisation by high frequency phonons at helium temperatures. Experience in one or more of the following areas would be an advantage: low temperature solid state physics, laser techniques, defects in semi-conductors, and ion implantation. Starting date 1 October 1985; salary on the first point of Research 1A scale (£7,520 - £12,150 under review).

Further particulars and application form available from the Establishment Office (quoting reference L2281B), University House, Bailrigg, Lancaster LA1 4YW where applications (five copies) naming three referees should be sent to arrive not later than 31 August 1985. (7359)O

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STUDENTSHIPS

UNIVERSITY OF NOTTINGHAM MEDICAL SCHOOL

DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY
FRAME RESEARCH STUDENTSHIPS

Applications are invited for two AME-sponsored studentships for research into the metabolism and toxicity of xenobiotics in mammalian cultured cells. Each studentship will be tenable for up to three years from

October 1985, with a view to obtaining the degree of PhD. Candidates should hold a first or upper second class honours degree in Pharmacology, Biochemistry or a related subject. The terms and conditions of the studentships will be similar to those offered by the MRC/SERC.

Applications, including a curriculum vitae and the names of two referees, should be sent to Dr J Fry, Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH (telephone No 0602-700111 ext 97) from whom further information may be obtained. Applications should reach Dr Fry by the 31st August 1985. (7305)F

ROBERT GORDON'S INSTITUTE OF TECHNOLOGY

School of Nutritional Science

RESEARCH STUDENTSHIPS

Applications are invited from suitably qualified persons for Research Studentships in the above School.

Subject areas include: (a) Studies of the metabolic activity of human and bacterial populations. (b) Nutritional education. (c) Trace element metabolism. (d) Investigations of lipid metabolism.

Application forms from Personnel Department, Robert Gordon's Institute of Technology, Schoolhill, Aberdeen, AB9 1FR. (7330)F

THE MACAULAY INSTITUTE FOR SOIL RESEARCH

Department of Soil Organic Chemistry

RESEARCH STUDENTSHIP

Applications are invited for a 3-year Research Studentship, funded by the whisky industry, to study the involvement of copper in the whisky distillery process, the nature of copper complexes formed, their relation to whisky flavour and their removal from waste products. The holder of the studentship will be registered for degree of Doctor of Philosophy of the University of Aberdeen.

Further information may be obtained from The Secretary, The Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen, AB9 2QJ, to whom applications giving the names and addresses of three referees should be sent by 26 August 1985.

Quote Ref SWR/1/85 (7318)F

ASSISTANTSHIPS

UNIVERSITY OF OXFORD

NUFFIELD DEPARTMENT OF ORTHOPAEDIC SURGERY
MRC Bone Laboratory

POSTDOCTORAL RESEARCH ASSISTANTSHIP

Applications are invited for the above post supported by a grant from the National Fund for Research into Crippling Diseases to investigate the basic physiological defects that result in crippling conditions of soft tissue ossification. The appointment will be for 28 months from October 1st, 1985. Initial salary will be within the range £7,520 — £9,860 pa on the 1A scale. Applicants should have experience in biochemical and cell biological research, preferably related to bone tissue metabolism.

Applications with full curriculum vitae and the names and addresses of three referees to Dr J T Triffitt, MRC Bone Research Laboratory, Nuffield Orthopaedic Centre, Oxford OX3 7LD, as soon as possible. (7315)P

UNIVERSITY OF STRATHCLYDE

DEPARTMENT OF PHYSICS

Applications are invited for a three year

RESEARCH ASSISTANTSHIP

for theoretical and computational modelling of dynamical instabilities in lasers and related optical systems. Models of self-pulsing and chaotic oscillation will be developed for qualitative and quantitative comparison with experimental work at a number of European laboratories. Considerable computational experience with at least one of laser dynamics, optical chaos, transverse mode coupling and density matrix analysis. A dedicated 32-bit supermicro, with powerful memory, graphics and language facilities available, with network access to large machines as required.

Salary on Range 1A (£7,520 — £12,150 per annum) USS benefit

Applications (quote Ref: R46/85) with full curriculum vitae and the names and addresses of three referees should be sent to Professor B Henderson, Department of Physics, John Anderson Building, University of Strathclyde, 107 Rottenrow, Glasgow G4 0NG.

Closing date for applications: 23 August 1985. (7326)P

Continued on page 24

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FELLOWSHIPS

UNIVERSITY OF READING

DEPARTMENT OF MICROBIOLOGY

Post-Doctoral Research Fellow

Applications are invited for this fixed three year Fellowship supported by MRC to commence as soon as possible after 1 October 1985. The project is to study the functions of the genome of polio and other picornaviruses using site-directed mutagenesis of infectious, cloned cDNAs. The investigation forms part of a larger programme concerned with the development of new prophylactic and therapeutic agents against medically important picornaviruses. Previous experience of recombinant DNA techniques and/or of virology would be an advantage.

Starting salary up to £8,920 per annum (under review).

Candidates (PhD) should apply for application forms, quoting Ref. R34A to Personnel Officer, University of Reading, Whiteknights, P O Box 217, Reading RG6 2AH, telephone (0734) 875123 ext 233. Dr J W Almond, currently at the University of Leicester, is professor elect of the Department of Microbiology at the University of Reading. (7321)C

THE ROYAL SOCIETY GUEST RESEARCH FELLOWS

The Council of the Royal Society invites applications for the support of Guest Research Fellows. The object of this scheme, established in 1980, is to assist outstanding leaders in scientific research in the United Kingdom to invite as guests scientists of proven ability, normally resident overseas, to come and work with them for periods of between four months and one year. The aim is to provide for research needs which can best be met by such international collaboration, and proposed guests should be key workers, normally already holding established posts, who would bring unique expertise to their hosts in the United Kingdom. Emphasis in selection will be on the abilities and field of research of the applicant working in the United Kingdom and on those special contributions to the applicant's research which would be provided by the visitor and are not available in the United Kingdom.

Applicants must be working in departments of science (including agriculture, medicine, mathematics, engineering and technology) in universities or research institutions in the United Kingdom. Direct applications from intending guests from overseas are not acceptable.

Applications should be made on forms to be obtained from the Executive Secretary (GRF/ADH), The Royal Society, 6 Carlton House Terrace, London, SW1Y 5AG, and returned by 30 September 1985. (7304)E

UNIVERSITY OF MARBURG

MOLECULAR BIOLOGIST or PROTEIN CHEMIST

Applications are invited for POST-DOCTORAL

RESEARCH FELLOWSHIPS

funded for three to five years starting September 85 or January 86 (Salary according to BAT II/IIIa/b). The group focus on hormonal regulation of gene expression in vertebrates. Applicants should have experience in either molecular biology, microinjection or protein chemistry.

Applications including c.v. and the names of two referees should be sent to: Prof. M. Beato, Institut für Molekularbiologie, Emil-Mannkopff-Str. 1, D-3550 Marburg, West Germany. (W2100)E

POSTDOCTORAL FELLOWS (3)

Postdoctoral fellowships (3) are available to study the molecular basis of human disorders, in particular Cystic Fibrosis, Fanconi anemia and Multiple sclerosis. Individuals wishing to apply their training in molecular biology to problems of human genetic disease are especially encouraged to apply.

Salaries are in the range of \$20,000 (CAN) and appointments are for 2 to 3 years.

Send letters of application and curriculum to Dr M Buchwald, Department of Genetics or Dr J R Riordan, Department of Biochemistry, Hospital for Sick Children, 555 University Avenue, Toronto, Ontario, M5G 1X8 Canada. (NW114)E

UNIVERSITY OF LEICESTER

DEPARTMENT OF BIOCHEMISTRY POST-DOCTORAL FELLOW

Applications are invited for a CRC funded post-doctoral fellowship to work on the analysis of gene expression in rectal and breast carcinomas using in situ hybridization to mRNA in tissue sections. The work on rectal carcinoma will be concerned with the cellular localization and levels of mRNA's for the basement membrane proteins laminin and fibronectin.

The project on breast carcinoma will be concerned with the cellular localization of transcripts for a number of (onco) genes found to be differentially expressed in breast carcinoma. Whilst it is desirable that applicants have experience in recombinant DNA technology, it is by no means essential as the Department of Biochemistry has in depth expertise in this area of research. The position is for 2 years and is available immediately. Salaries within the range £7,520 to £12,150 depending upon age and experience.

Applications, including a curriculum vitae and the names of two referees, should be sent to Dr D R Critchley, Department of Biochemistry, University of Leicester, University Road, Leicester, LE1 7RH, from whom further details are available (0533 551234 x 387) (7340)E

WORKSHOPS

Deutsche Gesellschaft für Biophysik
**Workshop on
 Nucleocytoplasmic Transport**

September 26–28, 1985

Deutsches Krebsforschungszentrum, Heidelberg, F.R.G.
 Organized by R. Peters and M. Trendelenburg

Considerable progress has been made recently in nuclear targeting of oncoviral and karyophilic proteins, of steroid receptors and ribonucleoproteins, in the structure, biochemistry and assembly of the nuclear envelope, in transcript organisation, and in physical techniques for measuring molecular transport in single cells. By bringing together scientists of various disciplines the workshop will reassess the potential role of nucleocytoplasmic transport for gene expression, cell cycle and growth control. The workshop includes lectures, poster sessions and technical demonstrations. Sessions and chairpersons or speakers so far accepted:

Nuclear envelope

Blobel (New York), Gerace (Baltimore), Krohne (Heidelberg), Milligan (Heidelberg), Newport (San Diego)

Laser fluorescence microscopy

Axelrod (Michigan), Garland (Bedford), Peters (Frankfurt), Ware (Syracuse)

Video microscopy and cell biological techniques

Allen (Hanover), Edstrom (Heidelberg), Feldherr (Gainesville), Neuhaus (Ladenburg), Trendelenburg (Heidelberg)

Karyophilic proteins

Burglin (Basel), Franke (Heidelberg), Hall (San Francisco), Kleinschmidt (Heidelberg), Laskey (Cambridge)

Ribonucleoproteins

Brachet (Brussels), Daneholt (Stockholm), Mattaj (Basel), Scheer (Heidelberg)

Steroid receptors

Dierich (Strasbourg), Greene (Detroit), Schutz (Heidelberg)

Oncoviruses

Colman (Coventry), Gissman (Heidelberg), Graßmann (Berlin), A. E. Smith (Framingham)

Poster presentations: Submit an abstract of one page maximum

Registration fee: US \$75 or DM250 by check of money order

Deadline: August 15, 1985.

Address for registration, accommodation and poster abstracts:

Workshop secretariat, Mrs B. Schulz

Max-Planck-Institut für Biophysik

Heinrich-Hoffman-Str. 7, 6000 Frankfurt 71 (W2098)V

GRANTS & SCHOLARSHIPS

**THE UNITED MEDICAL
 AND DENTAL SCHOOLS**

St Thomas's Campus
 Department of Obstetrics
 Department of Pharmacology
 Two PhD Scholarships

One scholarship is in the field of neuroendocrinology; this project will involve studies of the control of the synthesis and release of hypothalamic releasing factors. The other scholarship concerns transcriptional

changes in diabetes; this project involves the characterisation of altered genomic products in liver and pancreas in diabetic animals and the effects of insulin and anti-diabetic drugs

Applications in the form of a curriculum vitae and the names and addresses of two referees should be sent no later than 14 September 1985 to the Secretary, Department of Pharmacology, St Thomas's Hospital Medical School, London SE1 7EH (7350)H

ASSISTANTSHIPS CONTINUED

**UNIVERSITY OF LONDON
 British Postgraduate Medical
 Federation**

The Cardiothoracic Institute

**RESEARCH
 ASSISTANTSHIP IN
 MEMBRANE BIOLOGY**

Applications are invited for a 3-year BHF supported research assistantship commencing in October, 1985. Candidates should possess a first or upper second class honours degree in an appropriate subject and will be expected to register for a PhD. The research project will involve the

study of the mechanism of ion conduction through cardiac membrane channels following their incorporation into planar phospholipid bilayers.

Salary on University of London Research Assistant scale IB. Post supernannuable under USS.

Further details are available from Dr A. Williams (01-486-3043).

Applications to include full curriculum vitae and names of three referees should reach the Secretary, Cardiothoracic Institute, Fulham Road, London SW3 6HP by 27 August 1985. (7361)P

CONFERENCES & COURSES

**FIRST INTERNATIONAL CONFERENCE
 OF ANTICANCER RESEARCH**

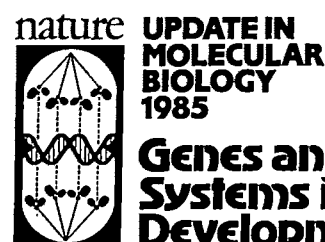
26–30 October 1985, Loutraki, Greece

Topics: Combination Chemotherapy, New Anticancer Agents: Development and Evaluation, Tumor Makers, Receptors, Monoclonal Antibodies in Diagnosis and Treatment of Cancer and Leukemia: Carcinogenesis (Mechanism, Environment, Nutrition) Invasion and Metastasis, Tumor Angiogenesis, Differentiation and Cancer Biology of Human Glioma Oncogenes, Leukemia and Lymphoma: Recent Advances, Strategies in the Development of New Tumor Markers, Chromosomes and Cancer, The Foundation of the International Institute of Anticancer Research.

Deadline for Late Registration and Submission of Abstracts: 10 September 1985.

Information: Dr John Delinassios, Anticancer Research, 5 Argypoulou Street, Kato Patissia, Athens GR-111 45, Greece. (W2106)C

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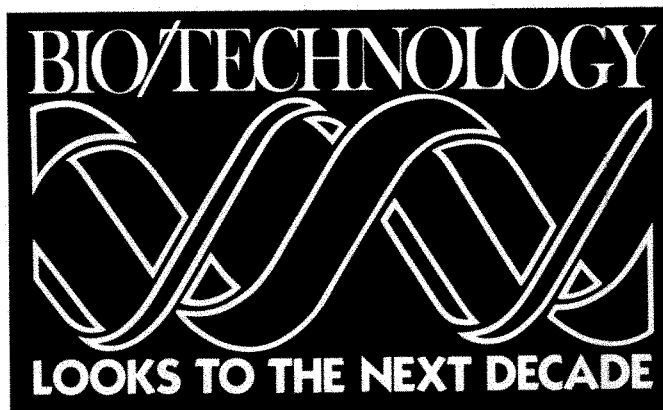
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Goeddel, Director, Molecular Biology, Genentech
Jan Borlaug, CIMMYT, Mexico City and Director, Dept. of Soil and Crop Science, A&M

JANUARY 20—AFTERNOON

Seminar in Molecular Biology

David Goeddel, Co-Chairman
David Flavell, Co-Chairman
Colby, Director, Technology Development, Triton Biosciences

Seminar in Agricultural Biotechnology

Jan Borlaug, Chairman
Art Fraley, Manager of Plant Molecular Biology, Monsanto
Jim Evans, Vice President for Research, Plant Technology, Inc.
Les Arntzen, Associate Director of Central Research and Development, E.I. du Pont de Nemours and Co.

JANUARY 21—MORNING

Commercial Production Processes

Speakers:
John Lilly, Professor of Chemical Engineering, University College London
Art Herschberg, Department of Process Development, Genentech
Yukio Sugino, Director, Biotechnology Labs, Sankyo Chemical, Japan
Ephraim Katchalski-Katzir, Professor of Physics and Biotechnology, Tel Aviv University, Israel

JANUARY 21—AFTERNOON

Seminar in Scale Up

John Lilly, Co-Chairman
Art Herschberg, Co-Chairman
John Buckland, Director, Computerized Pilot Plant, Merck, Sharp, & Dohme

Stanley Tarnowski, Research Section Chief, Dept. of Bioseparations, Hoffmann-La Roche

Seminar in Fermentation

Yukio Sugino, Chairman
Arnold Demain, Director of Laboratory of Fermentation and Microbiology, MIT
Behzad Khosrovi, Senior Director, Process and Product Development, Cetus Corp.
William Tolbert, Research Fellow, Monsanto

Seminar in Immobilization & Biocatalysis

Ephraim Katchalski-Katzir, Chairman
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Emil Thomas Kaiser, Haggerty Professor, Rockefeller University
George Poste, Vice President and Director of R&D, Smith Kline & French

JANUARY 22—AFTERNOON

Seminar in Vaccine Development

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Mark Greene, Professor, Dept. of Pathology, Harvard Medical School

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Gregory Petsko, Professor, Dept. of Chemistry, MIT

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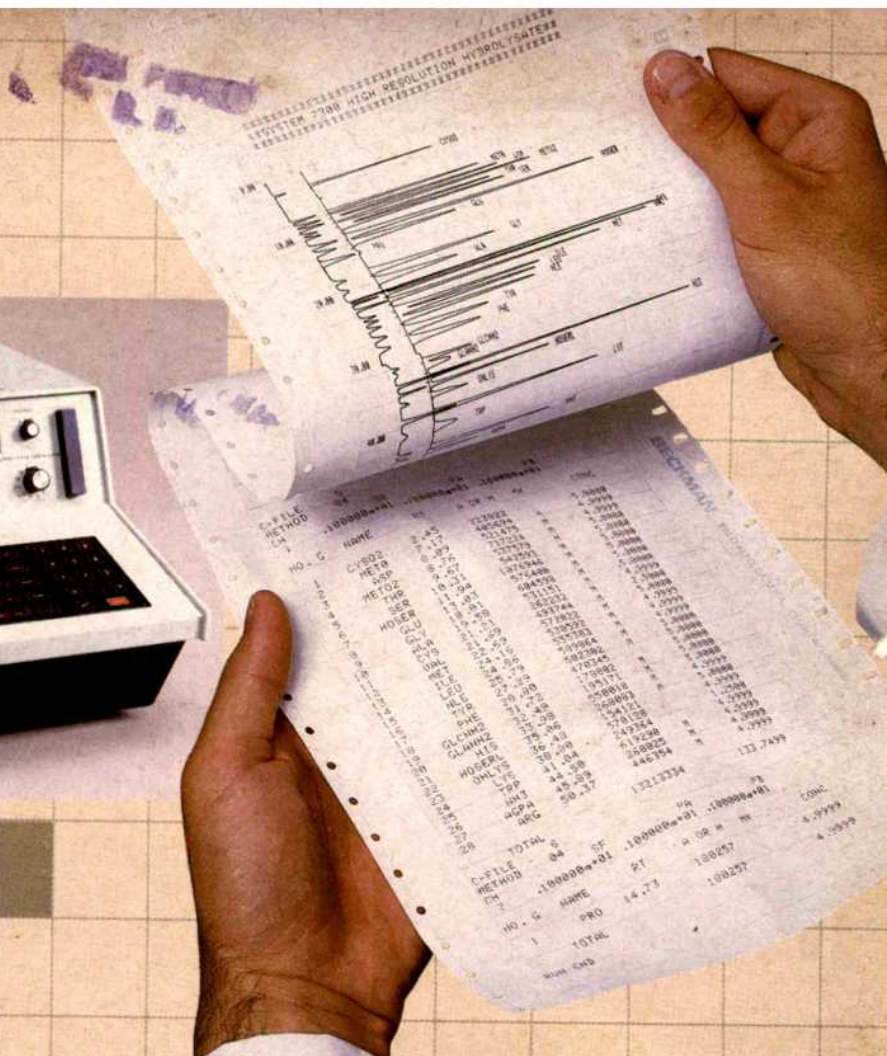
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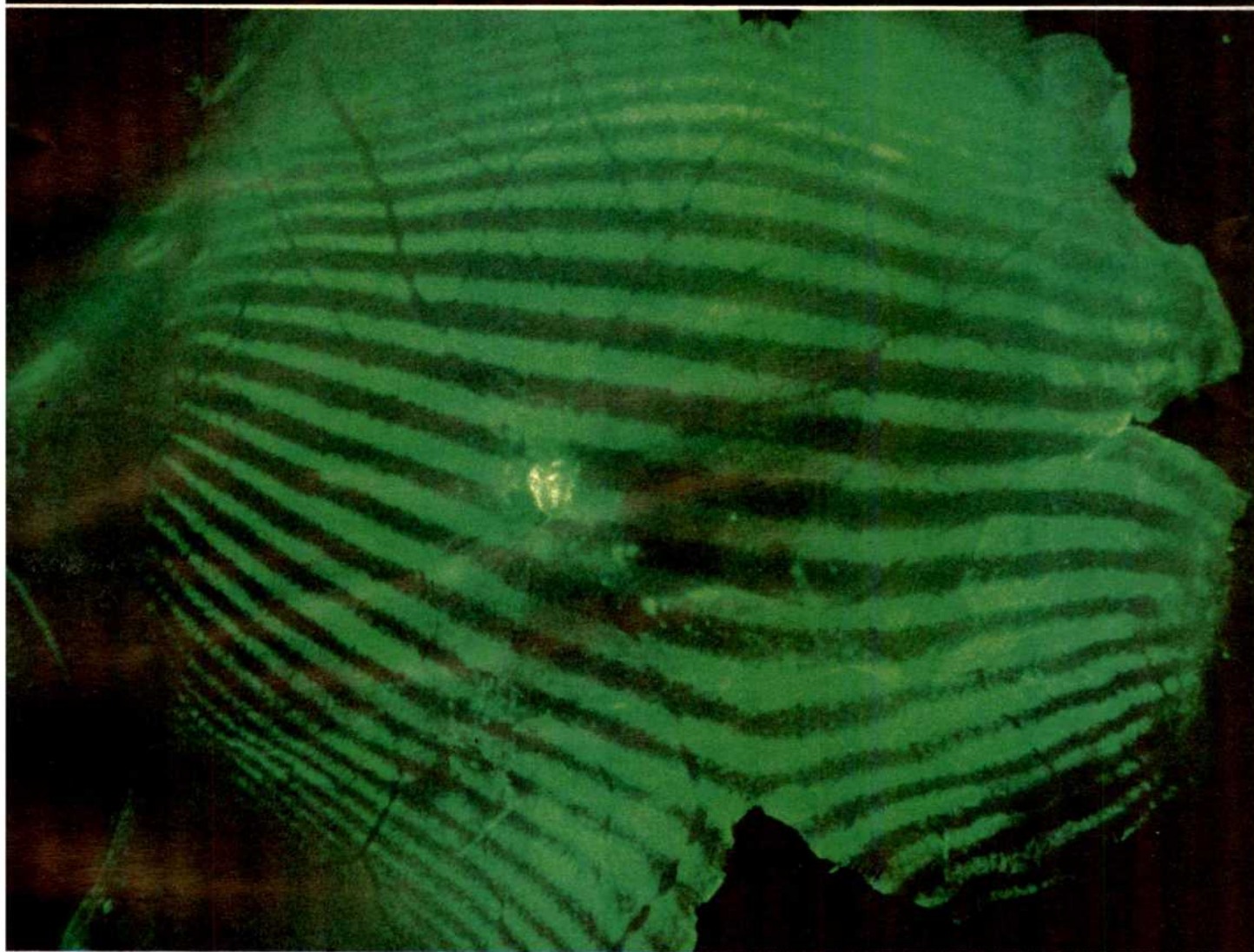
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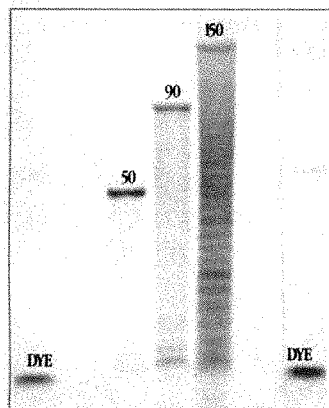
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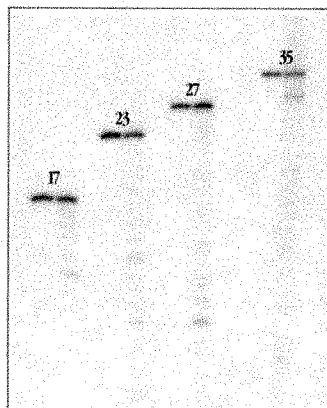


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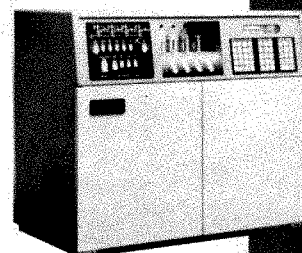
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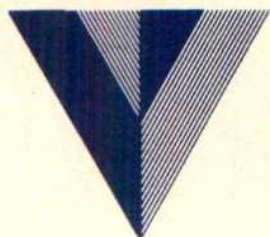
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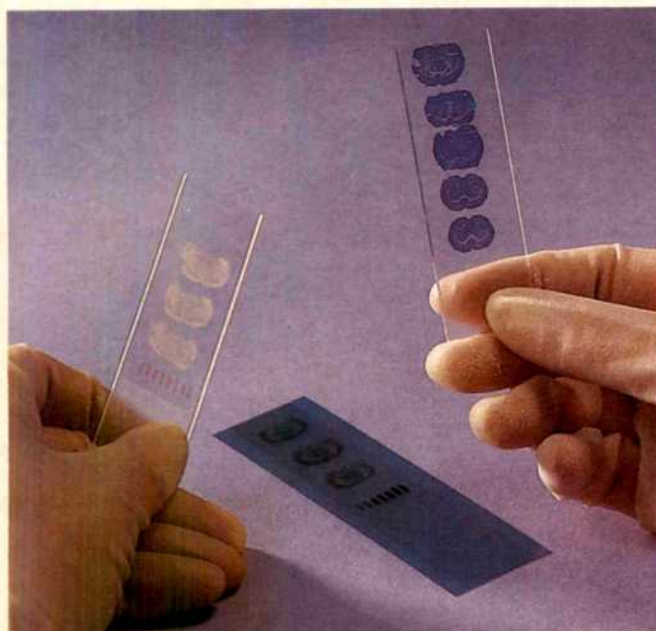


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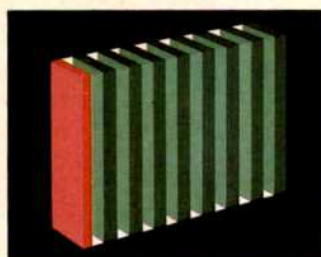
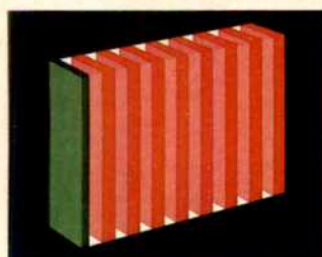


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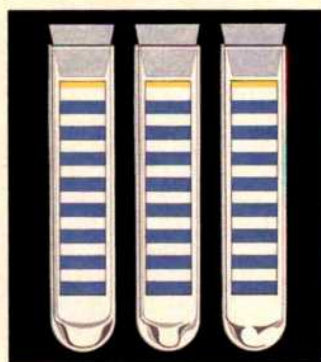
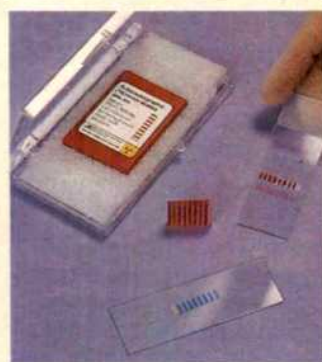
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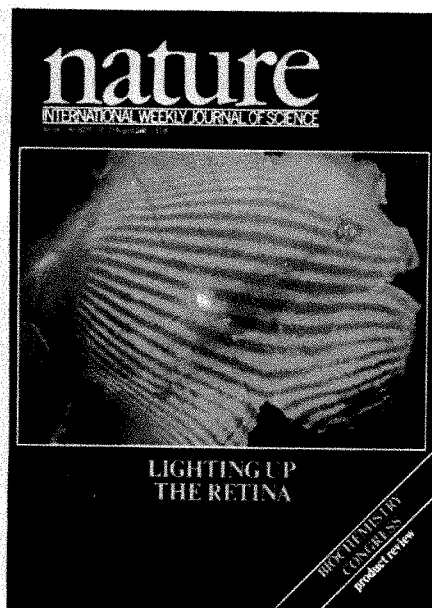
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A whole mount of a retina from a mouse exposed to a stationary visual pattern for 8 minutes and reacted with RT97, an antibody against light-activated sites. This new way of studying photoreceptor function is described on page 630.

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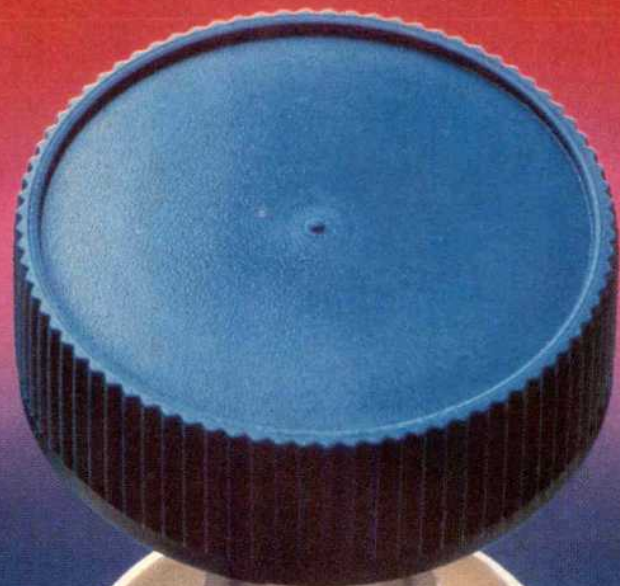
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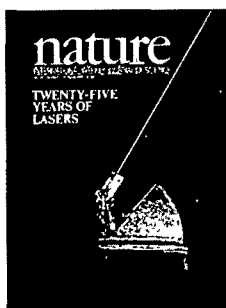
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K. Hayashi, N. Sakamoto

Dynamic Analysis of Enzyme Systems

An Introduction

1985. Approx. 350 pages
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The dynamic analysis by computer simulation presented in this work will furnish biochemical researchers with a means for better understanding the *in vivo* behavior of an enzymatic reaction and its regulation at the molecular level. The analysis can also be applied to systems used in biochemical and biomedical engineering.

Population Genetics and Molecular Evolution

Editors: T. Ohta, K. Aoki

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Editors: K.-H. Neumann, W. Barz, E. Reinhard

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T. L. Hill

Cooperativity Theory in Biochemistry

Steady-State and Equilibrium Systems

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In this book, the author gives a unified treatment of the statistical thermodynamics of cooperative biological systems. The aim is to introduce, by a very large number of examples, methods that can be used to treat cooperativity in both steady-state and equilibrium systems.

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P. Dustin

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Computers for British academics

The provision of computers in British universities has been generous while the rest of the system has been starved. The government should put computers on an equal footing with other equipment.

How can a university system (the British) generally supposed to be on the brink of collapse from penury be advocating expenditure of £47.5 million on the provision of supercomputers as a service for academic research? This will be one reaction to the report published this week (see p. 569). Indeed, many readers of the report will be surprised to learn that British universities already have access to two machines in the supercomputer class and that, moreover, there is a dedicated data transmission network linking all British universities with each others' machines and the two supercomputers. The explanation is that since the early 1970s, when the organization called the Computer Board was established, computing facilities at British universities have been dealt with separately from the other costs of academic institutions, and generously as well. During the past few years, funds in excess of £30 million a year have passed through the Computer Board's hands. Although the board, like the University Grants Committee, is a creature of the Department of Education and Science, it seems to have escaped lightly in the general rush to cut. The result is that British universities have reasonably good access to computing facilities. That is as it should be.

It is also right and proper that plans should be made now to keep the network up to scratch. Last week's report is both persuasive and arresting. The chief recommendation is that a decision should be made now to order a more advanced Cray machine, to be installed by 1988, and that the interval should be used for improving the data network to handle the extra flow of data. There are also sensible schemes for installing parallel processing equipment locally. Then, the committee argues, a decision should be made not later than the end of the decade on the specification of a second supercomputer specifically for academic use. There is the strongest possible reason why the British government (which will ultimately have to pay) should accept the committee's argument. Grumbles from elsewhere in the university system that £47 million could be better spent in different ways should not be allowed to carry too much weight. Even a university system generally in decline should be allowed to do some things excellently.

That should be the immediate response to a generally sensible report. The weakness of the committee's argument is chiefly administrative. That there should be a peer-review procedure for deciding who should have the right to bid for time on such supercomputer capacity as there may be makes sense. The idea that there should be a second tier of assessment to decide which problems have solutions of practical utility is more dubious. But the new proposal that there should be an entirely new board (euphemism for committee) with exclusive responsibility for the machines that happen, just now, to be called supercomputers makes no sense at all. This is a recipe for setting up a new committee whenever the evolution of computer hardware throw

up a radical novel generation of machines

The reason why this week's committee includes among its sponsors not merely the Computer Board but the Advisory Board for the Research Councils and the University Grants Committee, is that the sums of money involved are conspicuous. The Computer Board itself cannot hope to continue raising the funds it needs for general improvements and also to purchase supercomputers. But academic computers have come to stay, and like other scientific equipment will go on getting more powerful and (not as quickly) more expensive. The Computer Board itself should be recognized as an anomaly and subsumed within a larger organization. □

Worries about deficits

The failure of the US Congress to put the government's deficit to rights is serious for us all.

How much will the US deficit be in the financial year beginning on 1 October? \$200,000 million, the equivalent of \$700 for each man, woman, child and alien in the United States? Or 10 per cent above or below that figure? That is the untidy question the US government (the administration and the Congress in mutual cowardliness) has left for the rest of us to worry about. By now, the world knows that President Ronald Reagan rejected at the end of last month the plan of his party allies in the US Senate to save \$45,000 million by means of an oil import tax and the two-yearly staggering of social security revaluations, the Congress in panic settled for a mixture of cuts on budget spending that the appropriations committees may think it politically prudent to ignore.

Professional newspapers such as this must in prudence not stray too far outside their terms of reference. Liberally minded readers will acknowledge that concern for the workings of the educational system is proper, if only because that is what delivers recruits to the profession. Politically contentious though it may often be, even arms control is a permissible subject, partly because it has technical content, partly because research would come to an end if the cause of hard-headed arms control should fail. But why the US deficit? Because that too has technical content. And because the dice the US government is now tumbling could as certainly bring science and the enlightenment to a halt as an outright nuclear war.

Here is how the catastrophe may come about. For the past century, the United States has proved itself to be the most innovative and liberal of the mercantile economies of the modern world. The result is that people have become willing to lend money to US enterprises, knowing that in such an adaptable economy, the borrowers will probably make better use of the money than they could themselves. Moreover, they are usually confident that if things go wrong, they can hope to get their money back. Part of the reason for this confidence is that the US government historically has made only small demands on the US money system.

The big change is the deficit, at \$200,000 million roughly four per cent of the Gross National Product of the United States. The paradoxical situation has arisen in which foreigners finance a large chunk of industrial development, while the government in

Biological manuscripts

Contributors are reminded that, with the transfer of the Biological Sciences Editor to the Washington office, they should in future send **four copies of all manuscripts** offered for publication either (as at present) to London or to Washington.

Washington borrows its \$200,000 million a year largely from its own citizens and corporations. Interest rates are higher than they need be, given general confidence in the United States and the low rate of inflation over the past few years. The result is that the US dollar is artificially high compared with other currencies, which means that important sections of US industry, from agriculture to manufacturing, are competitively disadvantaged.

That the present circumstances are unstable is demonstrated by the fluctuations on the international currency markets. The threat to the US banking system occasioned by more than \$50,000 million of overseas loans is technically a separate issue, but one that could precipitate a loss of confidence in the dollar. Otherwise, there are two distinct ways in which the international community will deal with the deficit if the US government does nothing much about it: either people overseas will decline to keep on lending to the United States unless interest rates are further increased, in which case industrial production will further decline, the loss of confidence may go so far as to cause a flight from the dollar, in which case the currency will fall to where it should be, but corporations in the United States will be strapped for the cash they need to finance development. That is how the rest of the world sees the problem. In the United States, the prevailing sense, by contrast, is that trading partners are taking unfair advantage of the strong dollar, whence the widespread cry for protection from the rigours of free trade. The biggest danger now is that the Congress will listen more attentively to these voices than to the need to cut the deficit. The consequence, protectionism and counter-protectionism, could be just as serious as straightforward collapse. □

Action to follow

The US Food and Drug Administration plans to be more energetic. Time will tell if it succeeds.

Ms Margaret Heckler, Secretary of Health and Human Services in the US administration and, now that Mr James Watt has left the Department of the Interior, one of its most eager publicists, has embarked on a herculean task — that of preparing the Food and Drug Administration (FDA) for “the challenges of the twenty-first century”. More strictly, Ms Heckler has delegated the task to Dr Frank Young, director of the agency for the past year. Late last month, the two of them made public what the FDA calls *A plan for action*, a judicious blend of good intentions and more distant but even better aspirations. The temptation to make fun of this ingenuous document should, however, be repressed. Since the days during the Chinese cultural revolution when it was customary for Chinese public agencies to confess the errors of their past ways, and promise to do better in the future, there has been nothing like FDA’s vision of what it will become.

The starting point is tangible enough, the new procedures for the approval of new drug applications (NDA), published in final form in February. The intention is to simplify and accelerate the process of approving new drugs, partly by making FDA’s requirements of manufacturers more explicit, partly by admitting evidence gathered outside the United States in support of applications for generic drugs, for example. Along the same lines, a new set of regulations about Investigational New Drugs (IND) is about to make its appearance. Sensible proposals like these may not entirely meet the promise that the drug approval process may be shortened significantly from the present standard interval of 7–10 years, but they may at least prevent the further erosion of performance occasioned, among other things, by the increased sophistication of the testing procedures it is now possible (and prudent) to expect of applicants.

From that point on, the action plan becomes a good deal more fanciful. One proposal is that manufacturers submitting evidence in support of NDAs should be allowed to do so electronically, direct from their computer to another at FDA, thus doing away with the need to hire a truck to deliver the 100,000 pages of evidence which on the average accompany each application. The snag is that FDA has not yet equipped itself to receive these

electronic messages, and will be able to do so only when it can cheese the funds from elsewhere in its budget. The scheme for deepening the range of expertise available to FDA by appointing at universities a number of FDA fellows who would divide their time between research and the giving of advice is similarly still only a sentence or two in the plan. But FDA’s proposals for further improving the process of data collection and analysis as part of its surveillance of drugs already in use should be largely a matter of good housekeeping, and thus well within its own control. □

Geostationary blues

Governments are perplexed about the diplomacy of geostationary satellites.

A WORLD Administrative Radio Conference (WARC) of the International Telecommunication Union (ITU) must rank with anything on the subject of Canada as the surest way for newspapers to lose readers. But responsible newspapers acknowledge that, damn it, from time to time, ITU’s WARC’s have to be written about. The WARC now in session in Geneva, for example, is important: it will make assignments lasting a decade or more on radio frequencies and orbital positions for telecommunications satellites. Science writers have had to use all their ingenuity to make the subject comprehensible and, they fondly hope, interesting. And the biggest obstacles to their hopes are the names of the organization and of the conference and the technical problems involved.

The geostationary orbit, the circular path 36,000 kilometres above the Earth from which satellites appear to hover fixed over the same spot, is the hardest. The *Financial Times* made it sound quite cosy: “the most popular place for communications satellites . . . the ring in space which is home to about 80 operational communications satellites”. The *New York Times* disdained all technicalities and called it simply “the satellite belt”. How to brighten the name of the conference and the specialized agency of the United Nations which is running it? *Variety*’s “**Geneva Confab**” cannot be bettered, but neither can it be imitated. Other papers had to make do with phrases like “a six-week international gathering”. The *New York Times* did not mention the sponsoring organization.

Popularization is all very well but overdramatization is not. The need to make WARC interesting has pushed the newspapers into stating its purpose as unsnarling the “traffic jam in the heavens” or the “congestion over the Equator”. That is wrong, but the concept of a traffic jam is a poor metaphor. All the articles in which it appeared have then had to back away, explaining that there can be no physical pile-up and that the chances of collision in a path 265,000 kilometres in circumference are nil. Signal interference could be a problem, but even that is soluble with re-use of frequencies, higher frequencies and time-division multiplexing.

The real reason the conference was called is that the developing countries demanded it at the 1979 WARC. The United States, Britain and other industrial countries fought hard against it, but India insisted and, in the end, they had to give in. The developing countries demanded that they must have guaranteed access to places in orbit when they are ready, and so do not want the best positions to have been pre-empted by the industrialized countries. Equity in orbit is the problem, not congestion, and it will be tackled at two WARC’s — this summer’s and the second session set for 1988. The United States is fighting advance reservations on the grounds that the practice wastes spectrum and discourages innovation. But it concedes that it has to do something to placate the developing world’s legitimate gripe about the industrialized world’s monopoly on the orbit. This grows out of ITU’s long-established policy of awarding all frequencies on a first-come, first-served basis. The poorer countries do not like that either. About 90 per cent of the usable frequencies in the radio spectrum are occupied by 10 per cent of the 150 member countries of the ITU. □

Gene therapy

NIH/FDA dispute likely to delay research

Washington

THE US National Institutes of Health (NIH) have reached an uneasy truce with the Food and Drug Administration (FDA) in a dispute over regulation of human gene therapy. NIH's Recombinant DNA Advisory Committee (RAC) has spent the past two years drawing up a list of "points to consider" in evaluating gene therapy proposals, but a recent attempt by FDA to muscle in on the act had threatened a further lengthy round of reviews before experimental treatments could commence. Experimental gene therapies are now expected to be ready for trials before the end of the year.

The first gene therapy proposals will probably entail treating either Lesch-Nyhan syndrome or adenosine deaminase deficiency, both rare inherited diseases, by infecting patients' bone marrow cells with a retrovirus carrying the gene the patient lacks; the cells would then be replaced in the patient. *Points to consider*, drafted by a group under LeRoy Walters of Georgetown University, has already been published for comment in the *Federal Register* and a final version was to have been approved in September. Two weeks ago, however, FDA exercised its new authority as the official "lead agency" for biotechnology regulation and proposed that guidelines on gene therapy or deliberate release of engineered microorganisms should be reviewed by the new Biotechnology Science Board, a body that is to coordinate all federal agencies' interests in biotechnology. Meanwhile, FDA, as lead agency, would take control in areas where jurisdictions overlapped.

The Biotechnology Science Board has as yet no members, however, and it has not even been officially chartered. Faced with abandoning the past two years' work and having to start again, Walters' group has cancelled its September meeting.

The meeting at which FDA staged its coup attempt took place in the absence of NIH director James Wyngaarden. On learning of the FDA proposals, Wyngaarden met with FDA commissioner Frank Young (long rumoured as a future assistant secretary of health and unofficial "biotechnology czar") and secured an agreement that NIH would go ahead with *Points to consider* on the understanding that it applied only to NIH-funded research. The document will now once again be published for comment. As the first gene therapy proposals are likely to come from NIH-funded researchers, this measure should at least ensure that someone will read them when they arrive.

There are some points of substance in

the FDA/NIH dispute, besides the squabble over jurisdiction. RAC's meetings are held entirely in public, except when proprietary information is being discussed; FDA, in contrast, conducts its reviews in private. Wyngaarden plays down the importance of the dispute and says the confusion of roles has now been clarified: he points out that once bone marrow cells have been removed from the body and treated with a virus, they technically become "biologics" and so within FDA's purview. Historically, however, FDA has tended to turn a blind eye to *bona fide* researchers performing, for example, experimental diagnostic tests; it is not going to be so lax with gene therapy. Researchers will apparently have to seek approval for experiments from both NIH and FDA.

French science

Xenophobia out of fashion

THIS may not be an auspicious time to speak of an international stance in French scientific and technological relations — after President François Mitterrand has said "non" to star wars research, and after France has pulled out of European advanced fighter-plane project — but this is just what the French council of ministers was doing last week.

In a brief communiqué, the council (the French cabinet) said it wished to "intensify European cooperation", notably in relation to the Eureka project for new European high technology products, and would "reinforce our scientific and technological relations with the United States and Japan".

French research students are also to be encouraged to do their PhDs in collaboration with a foreign university, foreign researchers are to be given a better welcome in France than hitherto, and French scientific diplomatic representation abroad is to be reorganized, the communiqué said.

What this means in practice, however, is difficult to determine. The bid to improve research relations with the United States and Japan was "95 per cent exhortation", according to a French official, as it applied to industry, "over which we have little control", despite French industry's high degree of nationalization. However the intention was clear — to encourage French companies to learn from the Japanese and the Americans how to make a profit out of research: how to apply scientific know-how. For the feeling is that in certain sectors of the French economy, the government may have succeeded in putting researchers in touch with industry —

FDA officials say that is exactly the kind of over-regulation they are seeking to avoid. There also are criticisms of the way the NIH group under Walters, which is a subcommittee of RAC is constituted. One FDA official says that somatic cell therapy (the only sort contemplated in the near future) is analogous to many other medical procedures and so should be regulated similarly, by technical experts rather than by lawyers and bioethicists such as are found in Walters' group.

Points to consider describes the documentation needed to support a proposal for gene therapy: structure of the DNA construct, its purity, details of preparation, expression and animal studies; it also outlines the need for informed consent, measures to protect patient confidentiality and accurate information for news media. RAC is already considering one proposal that would allow it to bow out of areas where another agency has assumed responsibility for regulation; under this proposal, however, NIH would decide for themselves which areas to surrender.

Tim Beardsley

but there is some puzzlement in industry about what to do next.

In relation to universities, last week's communiqué may have more impact "as they take note of what government says". Jean-Pierre Chevènement, as a member of cabinet, has given the communiqué his backing, and must implement its recommendations. Thus it is likely that there will now be pressure on universities to design PhD courses in partial collaboration with foreign universities. How this might be done will be up to the universities, but the "habilitation" system, where universities must apply regularly for specific approvals to run postgraduate courses, would give the minister considerable power to influence the course of events.

Equally, it will be up to Chevènement and minister of research Hubert Curien to implement the recommendation that foreign researchers visiting France should be given better conditions of service. This means better grants for research students visiting France and fewer bureaucratic obstacles for more senior visiting scientists — despite the fact that last year all government-funded research positions became civil service posts.

As for French scientific diplomats, who are thicker on the ground in many countries than those of any other nation, there will be an attempt to "rationalize" their distribution and training, and, according to Paris officials, to cut costs. Many small countries enjoy the presence of a French scientific diplomat even though there is little research: such countries may lose their science counsellor, while larger countries may get more. Robert Walgate

Ocean Drilling Project

Britain still seeks for membership

WITH six weeks to go before the deadline, Britain is still scrambling to find 40–50 per cent of the £2.5 million fee needed to resume membership in the Ocean Drilling Project (ODP). As the crunch nears, Dr John Bowman, secretary of the Natural Environment Research Council (NERC), says the drive to participate has intensified. But so far, the roughly £1 million needed to take Britain over the top has not materialized. Britain was a member of the previous United States-led Deep Sea Drilling Program, but has not joined ODP for lack of funds.

"There is no doubt anywhere", says Dr Bowman, "of the scientific merit of ODP." Geologist Joe Cann, closely connected with ODP since 1975, described the importance of the new phase of the project. During the drilling of new holes, data will be gathered by sophisticated logging instruments (see *Nature* 316, 8, 1985); the holes will also be used for long-term monitoring of continuing processes beneath the sea floor, rather than as mere sources of rock samples. One of the most significant of the planned ODP projects, according to Dr Cann, is to intercept the deposits formed at the submarine hot springs that spew zinc, iron and copper sulphides into the ocean.

Mr Ed Nickless of NERC claims that 100 per cent of the membership fee could follow from talks now going on between the Department of Energy (DOE) and the oil industry, but not everyone is as optimistic. DOE, according to Dr Cann, is interested only in funding the drilling of holes in British waters that can then provide stratigraphic data of immediate relevance to British oil interests. The oil industry, which would prefer to do its own exploratory drilling, supports ODP as a source of ideas, not drilled holes. But Dr Cann points to the number of strategic ideas gathered from previous ODP trips that have been turned into exploratory tools for the oil industry in an "impressively rapid time — 5–10 instead of the usual 20–30 year span".

Refusal to dig deep into pockets also results from competition. British Petroleum, says Cann, looks at its direct competitors, primarily Exxon, and sees no reason why it should fund its country's involvement in ODP when the US company makes no comparable sacrifice. Additional industry money, therefore, could well not become available without an arrangement about taxation that would allow both industry and government to save face.

Dr Bowman defends the government's position, saying the shortfall of £1 million is a bigger hurdle than it may seem, particularly to observers in the United States, Japan and France, countries "with a lot more money to spend on the physical sciences". Besides, he says, there are many reasons to fund scientific research other

than the data gathered. But this, according to Dr Cann, is the real problem with British funding for the physical sciences in general and ODP in particular.

One case in point is British involvement in Antarctic research. So that a strong presence can be maintained there, the budget of the British Antarctic Survey (BAS) has been doubled in three years, from £6 million in 1982 to £12.96 in 1985. The results, Dr Frank Laws, head of BAS, is quick to point out, are easy to find — more staff, building programmes, satellite communication, heavy-cargo operations and funding of university research.

Yet these large sums of money earmarked for Antarctica do not give BAS unusual independence from NERC, says Dr Bowman. BAS just happens to be the primary research group funded by NERC which is involved in Antarctica. Because of ODP's work in the Weddell Sea, moreover, Dr Bowman says that rechanneling some of the Antarctic funds towards ODP would be appropriate. That amount, according to Dr Laws, would be a "significant contribu-

tion" that would still "not seriously affect BAS operations. By the same test, says Bowman, any sums taken from BAS would come nowhere near the elusive sum needed for British ODP membership.

Fiddling with budgets, says Dr Cann, can only hide the real problem. He claims that ODP decisions "have not been taken on a scientific basis at all," that "no real scientific presentation" has allowed, for example "an analysis of the relative scientific importance of ODP and BAS". The compartmentalization and politicization of such budgetary decisions is a description supported by the Royal Society Report on Geophysics, which sees Britain often taking on the role of poor relation in international efforts such as ODP. "Somebody, somewhere", says Dr Cann, "should make an impartial judgement" about the relative importance of physical science research as well as about ODP.

If Britain fails to find the full membership fee by 1 October, efforts will continue to join at a later date. NERC has put the ODP fee as second in priority to university research in its requests to the Advisory Board for the Research Councils, which will adjudicate in late November or early December.

Elizabeth Collier

UK research

All change at research council

In what may seem the nick of time, Britain's largest research council, the Science and Engineering Research Council (SERC), has found a new chairman. Professor E. J. W. ("Bill") Mitchell, head of



the Clarendon Laboratory at the University of Oxford, will begin a five-year stint on 1 October, only a month after his predecessor, Sir John Kingman, leaves to become vice-chancellor of the University of Bristol. At the same time, no fewer than six members of the 17-strong council will be replaced, at the end of their four-year terms, by newcomers.

Professor Mitchell is a solid-state physicist with a reputation as a forceful admini-

nistrator. His succession as chairman of SERC has been rumoured for some months. As a member of SERC since 1982, he will know of the unfinished business on the council's agenda. There is still no decision on the future of British collaboration with CERN, the high-energy physics laboratory at Geneva, while two important issues (support for astronomy and biotechnology) have been delegated to working parties that are likely to report only later in the year. The council's overall corporate plan is still being worked on while there is no certainty about the way which funds will be divided between the five research councils next April.

The newcomers include Mr David Shore, technical director of APV Holdings, who will become chairman of SERC's engineering board; Dr Charles Reece, director of research and technology at ICI; Professor Malcolm Jeeves, a psychologist who is now vice-principal of St Andrew's University; Professor Brian Fender, vice-chancellor of Keele University and formerly director of the Institut Laue-Langevin, who will be chairman of SERC's science board; Professor Robert Wilson, professor of astronomy at University College, London; Professor Donald Perkins from the University of Oxford, a particle physicist who will become chairman of SERC's nuclear physics board, and Professor David Davies from the University of London, an optical fibre specialist.

Supercomputing

Meeting British academic needs

A CENTRAL facility for advanced research computing is recommended this week by the report of a working party chaired by Professor A. Forty from the University of Warwick and commissioned by the Advisory Board for the Research Councils (ABRC), the Computer Board for Universities and Research Councils and the University Grants Committee (UGC).

The report proposes that the central installation should consist of the most powerful supercomputer available; the existing joint academic network (called Janet) should be enhanced for good communications; there should be a distributed system of other forms of advanced research computing to enhance local resources; and there should be a national organization to ensure effective use of resources, industrial and international collaboration and to stimulate new developments.

The cost of implementing the proposals will be about £47.5 million between 1986 and 1991. This includes £15 million for installation of the Cray X-MP multiprocessor array system at the Rutherford Appleton Laboratory, £8 million for high-speed lines and switches and £16.5 million for special purpose computers such as developments of ICL's DAP and GEC's GRID systems, which use distributed arrays of processors to perform operations in parallel. Running costs will be about £1 million a year. Purchase of a further supercomputer should be considered in 1988.

The report also proposes the machinery for running the facility: a board to advise ABRC, UGC and the Computer Board on advanced research computing needs and to award fellowships; and a peer review system to regulate use of facilities.

ABRC, UGC and the Computer Board must now decide whether they agree with the proposals and, if so, whether to allocate the money. ABRC is waiting for the individual research council's reactions to the report.

Recent advances in computing — vectorization and "parallel architecture" (simultaneous similar operations) — mean that the Cray 1 is 200 times faster than the VAX superminicomputer and 3,000 times faster and cheaper to use than the Atlas, the British supercomputer of the 1960s. Scientists in Britain currently have access to two supercomputers, the Cray 15 in London and the Cyber 205 in Manchester, linked by Janet. The London centre is now oversubscribed and Manchester is reaching saturation point. (There are also two DAPs in London and Edinburgh.) These supercomputers are not the most powerful models at present available.

The Forty report says the situation is different in other countries. In the United

States, the National Science Foundation programme started in 1984 involves the setting up of four supercomputer research centres ultimately to be linked by a national communications network. The government provides \$7–13 million a year to each centre and an equivalent sum is provided by industry, institutions and individual states.

In Japan, the seven main universities have a "special relationship" with one of the three major computer manufacturers (Hitachi, NEC and Fujitsu). Tokyo University, for example, has a Hitachi 5-810/20 supercomputer and Kyoto University a Fujitsu VP1100, both having replaced scalar machines at no extra cost. Other European countries such as France and West Germany are rapidly installing supercomputers and providing coordinated research programmes.

The new report's assessment of British needs is based on a questionnaire sent to

more than 400 individuals and scientific organizations and a detailed review of each subject provided by specialists in the field. The survey conclusively shows the need for a wide range of supercomputing facilities in astronomy, physics, earth sciences, engineering, biological and biomedical sciences, economics and information science.

Apart from the rapid understanding of many diverse scientific processes, the report emphasizes the use of supercomputers in industrial applications such as design and testing of pharmaceutical products using simulations, simulation and testing of aircraft engines and oil field modelling.

According to the report, there are compelling reasons for a coordinated and ambitious plan for the development of supercomputers in research in Britain. Haste is urged if Britain is to remain a world leader in the computer arena. Whether the bodies that commissioned the survey will agree, and if so, where the facility will be built and developed, remains to be seen.

Maxine Clarke

European training

Commission plan for mobility

Brussels

A FURTHER boost is to be given by the European Economic Community (EEC) to cooperation between European industry and academic institutions. The European Commission is advocating an 80 million ECU (about £44 million) four-year programme to stimulate on-the-job advanced training for students in companies in European countries other than their own. The scheme will also support exchanges between academics and those employed in new technology industries.

Later this year, the Commission hopes to persuade the ten ministers to agree to the four-year initial phase (1986-89) of the COMETT programme (Community in Education and Training for Technology).

Following in the steps of other Community initiatives such as ESPRIT, BRITE, the stimulation programme steered by the CODEST committee and the plan for transnational development of support in innovation and technology transfer, the programme will concentrate on new technologies, where it is felt that there is a particular need for more investment in training to keep up with rapidly changing technology.

The proposed programme would include exchange schemes between people in industry and universities as well as joint training projects between companies in different countries and the setting up of a European liaison network between university/industry training partnerships (UITPS).

Specifically, the Commission intends to start by offering some 2,000 six-month grants worth 4,000 ECU each for students

to train with a company in another Community country, with 3,000 places in 1988 and 5,000 in 1989.

A smaller number of grants (50 in 1987, to be doubled in 1988 and again in 1989) of 9,000 ECU would be available to university teaching staff willing to spend two or three terms in one year broadening their industrial experience in a company in another EEC country.

The Commission hopes there will be two kinds of joint training projects, one focusing on specific topics where skill is scarce and one designed to improve the flow of research results between industry and the universities. The Commission is prepared to pay 35 per cent of the cost up to a maximum of 500,000 ECU. It is also prepared to cover up to half the cost (a maximum of 400,000 ECU) for multilateral university/industry projects aimed at using new technologies in education and training itself. The COMETT team would also carry out a feasibility study for a European technological open university.

Some Community money would also be set aside (50,000 ECU a year) to cover half the running costs of promoting links between university/industry training partnerships, forming a Community-wide network coordinated by the Commission that would link up 40 UITPS in 1987 and 150 by 1989.

Finally, the Commission would also sponsor round-table meetings to bring industry and university together and would set up a database on cooperation, monitoring and analysis of Community trends and issues in advanced training.

Anna Lubinska

Space physics

NASA told to be more active

Washington

THE US National Research Council has told the National Aeronautics and Space Administration (NASA) to reverse the "long-term decline" in Solar System space physics, and has helpfully prescribed an ambitious series of projects to accomplish the task. The council's report* on the subject urges NASA to give highest priority to the International Solar Terrestrial Physics programme (ISTP), for which launches should start in 1989.

This influential support for ISTP should be music to the ears of NASA's partners in the project, Japan and the European Space Agency (ESA); three years ago, NASA infuriated European scientists by reneging on a promise to supply a satellite for the International Solar Polar Mission (now known as Ulysses). NASA officials now say there is "a reasonably good chance" that the agency will include funds to start work on ISTP in its budget request for fiscal year 1987 which NASA will finalize by 15 September.

The other two missions vying with ISTP for inclusion as a 1987 "new start" are

TOPEX (a satellite to measure ocean topography) and CRAF (Comet Rendezvous and Asteroid Flyby); the latest betting at NASA headquarters is that TOPEX and either CRAF or ISTP will be approved. In addition, the 1987 budget request is likely to include work on the mirror for AXAF, the Advanced X-Ray Astronomy Facility. By developing AXAF in stages, NASA hopes to avoid the embarrassing cost over-runs that plagued development of the Hubble Space Telescope.

The National Research Council Report, chaired by Stamatis M. Krimigis of the Applied Physics Laboratory at Johns Hopkins University, assumed a plausible (if hopeful) budget for Solar System space science of \$400 million by 1987 (in 1985 dollars); the current figure is \$300 million. NASA officials say the projects described in Krimigis's report are well justified and that it will be for Congress to decide how many will be funded.

Krimigis's report gives its blessing to the Upper Atmosphere Research Satellite, now being developed, and urges "immedi-

ate" funding for development of the Solar Optical Telescope, a 1.25-metre-aperture instrument that would form the basis of a solar observatory on the space station. It also wants to see a start on a long talked-about solar probe that would approach the Sun to within 4 solar radii; work should begin immediately on a heat shield for the mission in time for a possible launch in 1995. Looking further to the future, the committee gives its blessing to a solar polar orbiter that could be launched by the end of the century if a suitable propulsion system is developed.

Besides the highly visible major missions, the research council endorses an expansion of ground-based modelling and theoretical work in plasma physics; one goal is to provide an account of how disturbances on the Sun affect the heliosphere and thence the magnetospheres, ionospheres and upper atmospheres of the Earth and planets. There should also be a series of Explorer payloads, according to the committee, starting in the 1990s, to investigate Solar System space physics.

Besides being extremely colourful, the report is admirably specific in its recommendations. The committee's purpose might well be to avoid the fate of the 1980 Kennel Report on the same subject, on which the committee plaintively notes there has been "no significant progress" because of budget constraints.

Tim Beardsley

*An implementation plan for priorities in Solar-System space physics (Executive summary) Committee on Solar and Space Physics, National Research Council, 1985.

European research

EEC plans risk management

Brussels

THE European Commission has suggested to ministers of the European Economic Community (EEC) that 15 million ECU (European Currency Units, 1 ECU = £0.60) be provided over the next five years for research into the prevention, prediction and management of industrial accidents involving chemicals such as those that occurred in Seveso, Italy, in 1976 and last December in Bhopal, India.

Part of the new five-year 150 million ECU programme that also includes environmental protection and climatology, contracts on major technical hazards, to which the Community would contribute half the costs, will complement research already under way at the commission's own Joint Research Centre. These contracts will involve both industry and research institutes. It is hoped also to involve researchers from outside the EEC.

The aim will be to improve the scientific foundation of risk analysis by studying the physical and chemical phenomena of dispersion, runaway reactions and other causes of chemical accidents. There will also be an attempt to improve or replace hazardous processes, to improve safety technology, and to detect and mitigate high-risk situations. The Commission proposes that a start should be made on the transportation of bulk hazardous substances and on hazardous production processes.

The proposal would strengthen EEC

legislation sparked off by the Seveso disaster, when highly toxic dioxin was spewed accidentally over a north Italian town. The directive on the prevention of major industrial accidents came into force in 1981, but its implementation has posed difficulties for the member states.

In the environmental part of the research programme, for which 65 million ECU has been set aside, emphasis will be placed on health and ecological effects of pollution (particularly the deterioration of historic monuments), air, water and soil quality (including the impact of farming practices), waste, chemicals assessment (including the development of methods to avoid using vertebrates in toxicity tests) and noise. Emphasis will be put on advanced pollution abatement technology, in the hope that the outcome will also improve industrial competitiveness and employment prospects.

The Commission also intends to promote training in environmental sciences. Finally, 250 million ECU will be set aside for the second phase of a programme of long-term climate research. Work will continue on the physical basis of climate modelling, seasonal forecasting of European climates, climate sensitivity, effects of changes in atmospheric composition (via carbon dioxide and trace gases) and changes in land-surface properties, detection of climatic change and climatic impact on land, water, vegetation and marine resources.

Anna Lubinska

Polish protest (cont'd)

POLAND's unofficial Social Committee for Learning, SKN (*Nature* 314, 123; 1985) has been quick to respond to the enactment, on 25 July, of the government-sponsored amendments to the 1982 Higher Education Act. A copy of SKN's irregular news bulletin *Servis informacyjny SKN*, dated 28 July, presents a reprint of the official announcement of the new law with the "tentative summary" of the effect of the changes, prepared by the Main Council for Science and Technology, the official representative body of the universities.

In the opinion of the Main Council, "without embarking on a major discussion of the motives and grounds for the proposed changes, they are clearly aimed at the creation of a legal framework for the direct and centralized direction of all matters relating to higher education in Poland, not only in questions of its organization but also in a wide range of questions of cadre policy and scientific research, not excluding legalized para-censorship".

The SKN bulletin also notes, however, that in the opinion of Deputy Jarema Maciszewski, who presented the bill to the Sejm: "The proposed changes do not contravene the idea of university self-government, but simply restore its purely academic character".

Vera Rich

Japanese psychiatry

Mounting pressure for reform

Tokyo

JAPAN'S mental health system is once more under fire. Representatives of the International Commission of Jurists (ICJ) and the newly formed International Commission of Health Professionals (ICHP) who came to Japan on a fact-finding mission in May published their first report last week. It makes disturbing reading.

The present "structure and function of the Japanese mental health service" is summed up as "conducive to inappropriate forms of care and serious human rights violations on a significant scale". As examples, the report lists "unacceptable conditions of overcrowding and poor nutrition which may lead to physical deterioration of patients and high rates of mortality; physical abuse of patients; exploitation of patient labour; unjustifiable detention; the inability of hospitalized patients to communicate with friends and family members outside the hospital or to receive visits under reasonable conditions".

That description may seem more fitting to a prison camp than a place of care for the sick but there is, regrettably, evidence that such conditions have existed in Japan. The report does not, however, mention specific cases; its avowed aim is to comment on the "overall system" rather than on individual occurrences.

Had specific cases been taken up, there is no doubt that the Utsunomiya hospital would have been first on the list. The hospital became the subject of major national scandal last year. It seems that violence and confinement were regularly used to subdue patients, mail was censored, meetings with outsiders prevented, patients forced into labour, unqualified staff employed, money embezzled and patients admitted and detained illegally.

Such at least were the more minor allegations made against the hospital staff; the serious allegation was that two patients were beaten to death, one for trying to escape. The hospital superintendent is now in prison but the case is far from cleared up. Independent psychiatric examinations after the scandal resulted in 40 per cent of the hospital patients being discharged; but there are accusations that a significant number of patients are still being unnecessarily detained.

Within the past few weeks, similar accusations have been made at another hospital, the Otaki hospital in Chiba prefecture. Once again there are allegations that a patient has been beaten to death by hospital staff. And two patients hospitalized after family quarrels and kept for long periods in locked wards have been judged sane by independent psychiatrists.

Despite the publicity given to these incidents, it would be quite wrong to think of

them as typical; most doctors and hospital staff are conscientious and see these abuses as "isolated exceptions". But what the ICJ/ICHP report makes clear is that the present Japanese legal system makes it all too easy for abuses to occur, however conscientious the average doctor may be — without reform there will continue to be "isolated exceptions". The major driv-



ing force behind the hospital abuses is undoubtedly greed. More than 80 per cent of psychiatric beds are in private mental hospitals and huge profits can be made. The temptation to cut patient care and hospital costs is great, especially as government supervision is minimal.

The report makes a number of specific recommendations for reform. Most notably, it is suggested that all cases of "involuntary hospitalization" be subject to independent review. As a recent government survey has shown, 93 per cent of inpatients are confined against their will (against just 5 per cent in the United Kingdom) and 80 per cent are confined under Article 33 of the Mental Health Act. This article allows the superintendent of a mental hospital to commit a patient indefinitely without the patient's consent and without the opinion of an independent psychiatrist provided the person responsible for the patient gives consent.

Clearly, as hospital profits require full beds, it is all too easy for a hospital superintendent to be tempted to misuse his powers. Stories have even been circulated of hospitals rounding up tramps from the streets of Tokyo in order to fill their wards; although such stories have never been substantiated, the fact that serious newspapers publish them at all suggests that public confidence in mental institutions is not high.

The report further suggests that an independent review tribunal be established, mental hospitals be regularly inspected and patients have right of access to the tribunal. At present, Article 38 of the Mental Health Act provides that "the administrator of the hospital may set necessary limits to the action of any in-

mate...". This extraordinarily wide power given to the hospital administrator has allowed censoring of mail, and refusal of visits and independent psychiatric assessment. Some patients have virtually been turned into prisoners without rights.

The report sees the general thrust of reform as towards the "community care" style of psychiatric treatment now espoused (if not adequately supported) in the West: that means short periods of hospitalization, provision of out-clinics attached to health centres, sheltered "half-way" houses, home visits by health workers, patient clubs, help in finding employment and so on. Such measures contrast starkly with the present Japanese system of large hospitals located in remote regions where land is cheap and long hospitalization periods. And while mental hospital populations are declining in the United States and Britain, those in Japan are increasing.

The report does not seek, however, to impose external standards of care on Japan and repeatedly stresses that changes need to be adapted "to meet the needs of Japanese society". Nor does the report paint a universally black picture. Recognition is given to psychiatrists, health officials and concerned citizens who are already actively trying to bring about change, and mention is made of innovative mental health programmes already in existence.

Given the clear aim of the report to be constructive, the response of the Japanese government has been very disappointing, to say the least. All the government has done is to question whether the report was truly the unanimous opinion of ICJ and to dispute the view, presented in the ICJ/ICHP report, that a previous critical report from the World Health Organization had not led to any changes in Japan's mental health policy. The first question was based purely on rumour and was quickly dismissed by ICJ. The second appears to be mere nitpicking. Not one of the major recommendations of the WHO report has been implemented. Given that it was published in 1968, astonishingly little progress has been made.

Hopes for change in the future are not bright. Considerable stigma is still attached to the mentally ill and it is unlikely that public support for reform will grow rapidly. A number of hospitals have introduced reforms but few of them are in the private sector, and without government backing, change will be slow. The major impetus for reform seems to come from foreign critics. The problems of Japan's hospitals are, at least, becoming internationally known. One of Japan's foremost campaigners for reform of the Mental Health Act, lawyer Etsuro Totsuka, spoke for the first time at a meeting of the British reform group MIND a few weeks ago. The ICJ/ICHP report will be submitted to the UN Subcommittee on Human Rights in the near future. **Alan Anderson**

Catastrophism still unexplained

SIR—Despite Napier and Clube's frequent (and frequently changing) statements on the nature of terrestrial catastrophism and possible extraterrestrial causes, they were not the first to point out that the Sun's motion in the Galaxy might be the causative agent, as stated by John Maddox (*Nature* 315, 627; 1985). At least one earlier suggestion of this idea was a quite excellent paper by K.A. Innanen and colleagues entitled, "The interaction of the spiral density wave and the Sun's galactic orbit"¹ which appeared in 1978. That paper noted and displayed a figure showing the coincidence between galactic plane crossings and the boundaries between geological periods on the Earth.

Also, if I remember correctly, Napier and Clube² suggested that the important mechanism would be the Sun's passage through galactic spiral arms which happens every 250 Myr or so, as opposed to galactic plane crossings which occur every 33 Myr.

The periodicity in the terrestrial extinction record is certainly in grave doubt at this time. But I suggest that if there is any real periodicity it is most likely associated with a period of which we are already aware, that is the Sun's epicyclic z-motion about the galactic plane, rather than any of the artificial constructs we have seen suggested, such as the hypothetical death star or planet X. However, the problem of finding the extinction mechanism which might be associated with the Sun's epicyclic motion still remains unsolved.

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1 Innanen, K.A., Patrick, A.T. & Duley, W.W. *Astrophys Space Sci.* 57, 511–515 (1978)

2 Napier, W.M. & Clube, S.V.M. *Nature* 282, 455–459 (1979)

Conference costs

SIR—It cannot be good that British scientists are unable to attend conferences in their own field held in their own country, a situation that now prevails.

American colleagues report to me that they come to conferences in the United Kingdom in the hope of meeting their British counterparts, only to find that apart from invited speakers, British academics, both staff and graduate students, are largely missing.

The cause is simple: *money*. Conference fees are now enormous (to pay presumably for the air fares of the overseas speakers), and there was even an institution which ran a "hundredth birthday" symposium from which its own staff were excluded unless they paid a large fee! At the same time, in order to stay afloat, the universities that host the conferences are

charging "economic" (meaning swinging) rates for accommodation and meals, while they and other publicly funded institutions are unable to reimburse anything but the smallest sums for attendance at conferences. (My own institution now pays a maximum of £40 a year, barely the train fare to London, and this by national standards is generous.) Erosion in the real value of salaries makes it difficult, particularly for younger staff with mortgages and children, to find the several hundred pounds that a conference may cost out of their own funds. British scientists are thus deprived of the international contacts that they should have, and of much needed opportunities to keep abreast of their fields.

Remedies are not so simple, but here are two suggestions: as all universities are levying enormous accommodation charges on each others' staff, they are in effect taxing their own staff for carrying out a necessary part of their work. An immediate convention should be set up by the Committee of Vice-Chancellors and Principals allowing British academics to be charged for accommodation at cost when attending a meeting at another university in this country. Second, the Royal Society should consider extending its system of grants for attendance at conferences overseas to include attendance at conferences in the United Kingdom.

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Value-free science

SIR—The idea of value-free science has been defended by two of your correspondents over the past several months, Bernard D. Davis (*Nature* 311, 294; 1984 and 315, 176; 1985) and M. Hammerton (313, 343 and 315, 536; 1985). Unfortunately, they have not satisfactorily answered the points raised by Mark Diesendorf (313, 92 and 314, 666; 1985).

Davis's illustration of the risk of fallout from nuclear weapons provides an excellent illustration of the different ways in which values are embodied in science.

Research related to nuclear weapons and fallout comes overwhelmingly from the governments of nuclear weapons states. That means that these governments influence the direction of scientific development. After all, values are involved in choosing a particular facet of the world to study and about which to formulate knowledge.

Knowledge about nuclear weapons and fallout is selectively useful to the governments of nuclear weapons states because they are the ones who can hire experts to apply it. Community groups do not have much use for nuclear science. Scientific knowledge is value-laden because it is selectively useful to different social groups.

Knowledge about hazards from fallout has often been used to justify continued nuclear weapons testing, ignoring the fact that the "benefits" of testing accrue to governments and militaries while the hazards literally fall on many people who obtain no benefit at all. Much scientific knowledge is value-laden because it serves to justify policies and practices of certain groups. Another example is the setting of "acceptable" levels of exposure to radiation: acceptable to whom?

In the past, defenders of nuclear technology have often adopted the hypothesis that there is a threshold beneath which radiation exposure poses no risk to health. This illustrates how the content of scientific knowledge can embody values.

These and other points are elaborated and illustrated in my study *The Bias of Science*.

It is futile to try to argue away or exorcise values from science, since this only obscures the role of the social context of science. More appropriate is the aim of making the values open and apparent, so that the direction and use of science can be a subject for public debate.

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Paul Rosbaud

SIR—The Houghton Mifflin Company will publish in 1986 a book about Dr Paul Rosbaud, scientific adviser to Springer-Verlag in Berlin until 1945. After the Second World War, Rosbaud became associated with various scientific publishing activities in Britain until he died in 1963. Copies of letters, papers and so on associated with Rosbaud would be deeply appreciated by the undersigned. Particularly welcome would be any reminiscences about Rosbaud before 1946.

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Faith in God

SIR—In his review of Hugh Montefiore's book *The Probability of God* (*Nature* 315 353, 1985), John Maddox seems to imply that religious belief is almost some kind of moral and/or mental inadequacy. I would say rather that belief in God—any god—must ultimately be irrational, for it involves a step of faith. If belief were to be rational, that is, if the existence of God could be proved, then it would be meaningless, for surely a god whose existence can be proved by the activities of man is no god at all.

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Towards the quantum computer?

The Turing machine, an abstract computer conceived half a century ago as a test of what might be computable, has been overtaken by an idealized quantum computer, which also has drawbacks.

THE notion of the quantum computer means different things to different people. At one extreme are the hardware engineers, the people who behave (successfully) as if for every physical phenomenon there is at least one practical device. IBM's recent disappointments with Josephson junctions apart, these people know in their bones that the quantization of magnetic flux, as within closed loops of superconducting material, will one day be the basis of a memory storage element. The other extreme is the conceptual, typified by those who worry over the correspondence between the elementary units in which information is handled in computers and the quanta of the physical variables that characterize real physical systems.

A whole string of more substantial questions obviously suggest themselves. To what extent, for example, is the accuracy of a computer calculation limited by the deterministic uncertainty of quantum mechanics? Whatever the care with which signals are processed so as to encode a memory cell with either the digit zero or unity, the result cannot be entirely certain while the uncertainty principle remains in being. This is not a practical difficulty for the time being, while the scale of the most tightly-packed electronic components is measured in some hundreds of interatomic distances, but in due course the uncertainty principle must determine the limits to which it will make sense to carry very large-scale integration.

Meanwhile, but for a different purpose, Dr D. Deutsch from the Department of Astrophysics at the University of Oxford (*Proc. R. Soc. A* **400**, 97; 1985) has given what seems to be a way of describing the properties of a quantum computer which, among other things, has the virtue of showing the physical nature of the computing process. Quantum computers, it appears, are potentially more powerful in important ways than even that computational paradigm known as a Turing machine.

A Turing machine (first described by the late Dr Alan Turing nearly half a century ago) is, it will be recalled, a stripped-down computer, consisting only of an indefinitely long memory tape and a device (which would now be called a processor) whose state at any instant depends both on its previous state and on the most recently read item of information on the tape. On the assumption that the informa-

tion on the tape includes what would now be called the program as well as the input data, the pedagogical (some would say "heuristic") value of the Turing machine is to demonstrate the power or the limitations of the computing process, depending on one's point of view. Turing's purpose, in 1936, was to assert that such a machine, however rudimentary it might seem, could compute anything generally held to be computable. Deutsch, to be fair to J. Church (*Am. J. Math.* **58**, 435; 1936) calls this the Church-Turing principle.

Put like this, the principle sounds like a tautology. Deutsch argues that the tautology conceals a physical reality. The output datum of a computation will be a function of the input datum, so that the question of what may be held to be computable boils down to specifying the class of functions that can be realized by a Turing machine. Deutsch points out in passing that all that such a machine can do in practice is to make a map of the set of all the integers on itself, which is not exactly the same as defining the functional relationship between input and output (a point of some importance when so many people are interested in chaos, where a small change of an input variable may mean a very large change in the corresponding value of the output). More tellingly, Deutsch goes on to argue that the truth about the class of computable functions, or of conceivable algorithms, is that they are physically realizable. That mathematics is the handmaiden of physics is not an accident. And so Deutsch puts the "Church-Turing principle" in the form "Every finitely realizable physical system can be perfectly simulated by a universal model computing machine operating by finite means".

What kind of machine is that? Luckily, the formalism is not difficult, and can be put in words. The machine has a memory, which may in principle be infinite, but only one bit is involved at any step. There is a processor whose internal (quantum) state represents the progress of the computation. And there is an input/output tape which can move only one step (forwards or backwards) at a time, and whose position is an observable (in the sense of quantum mechanics). The evolution of the system as a whole from one step in the computation to the next is determined (as in ordinary wave mechanics) by the operation of some unitary operator on the state vector of the system lying within some appropriately catalogued Hilbert space. Although the dimen-

sionality of the problem will be dominated ordinarily by the number of memory cells, contributing that many powers of two, the problem is simplified because the elements of the unitary transformation matrix are themselves simplified by the requirement that the tape should move by only one step at a time, and that only one memory cell should be involved. One computer will differ from another in the structure of the unitary transforming matrix.

The formalism may be describable in words but the details show that the theory hangs together. Deutsch shows that a quantum computer (as defined) can carry out any computation of which a Turing machine is capable but that, because the elementary unitary transformation can mix together quantum states in ways that have no classical analogues, the universal quantum computing engine can yield results beyond the ken of classical machines.

For one thing, a quantum computer should be capable of generating true random numbers and not simulations thereof. (Proud custodians of new hardware who are convinced that their classical machines will generate random numbers for them had better read the 178 pages of *The Art of Computer Programming* Vol.2, in which D.E. Knuth explains why the programs purporting to give random numbers at the push of a button cannot be trusted.) Deutsch gives two sample programs. He goes on to show how to write a program (for a still non-existent quantum machine) to verify Bell's inequality about the essentially quantum character of quantum correlations. There are even a dozen lines of Algol 62 which simulate the Einstein-Podolski-Rosen experiment.

The interesting part of the argument concerns the interaction of the quantum computer with the outside world. To make this non-existent machine function, it is necessary that its initial state should be "prepared", in the sense of quantum mechanics, to be in some well-defined state, which is the same thing as saying that it has zero entropy. Deutsch himself points out that the third law of thermodynamics would make such a state inaccessible in finite time, goes on to say that the evolution in time of a small part of the Universe (such as a quantum computer) may be determined by a non-unitary transforming matrix and declares that it is of practical as well as of philosophical importance to learn what the rules are. Nobody will quarrel with that.

John Maddox

Molecular genetics

Introns continue to amaze

from Andy Flavell*

IN 1977 Joe Sambrook wrote a *News and Views* article in *Nature* entitled "Adenovirus Amazes at Cold Spring Harbor" in which he described the first experiments demonstrating RNA splicing¹. Since then we have learned that most eukaryotic and some prokaryotic genes contain intervening sequences (introns). Time has mellowed the initial astonishment but as we become accustomed to each new finding in this field, another fresh observation startles us out of our complacency. We have learned that some introns can remove themselves from precursor RNAs without recourse to proteins², others encode proteins which aid this process³ and many form circular excision products, some with 'strange' lariat structures^{4,5}. Those who thought that the flow of startling observations would diminish will have to reconsider in the light of two sets of results, one of which is reported on page 641 of this issue⁶.

A big problem for molecular evolutionists has been the question of why genes have evolved in this manner⁷. One major school has suggested that introns have existed as long as recognizable genes⁸⁻¹⁰ and initially represented the junctions between small units of genetic information. Long precursor RNAs could self-splice to create assortments of these units, successful combinations being rewarded in the normal Darwinian manner. There were early attempts to argue that introns were vestigial mobile elements which had been transposed into genes¹¹⁻¹³ but no evidence emerged to support this notion. Recent results on yeast mitochondrial introns may, however, provide hard evidence that introns are far from passive sequences, with some perhaps able to ensure their survival by transposition to new locations^{6,14,15}.

Michel and Lang now demonstrate sequence homology between certain fungal mitochondrial messenger RNA introns and the reverse transcriptase genes of retroviruses⁶. Reverse transcriptase is the key enzyme in the retroviral life cycle; it converts the viral RNA into DNA, which becomes incorporated into the host genome. Michel and Lang have compared the predicted amino-acid sequences of the long open reading frames of four class II introns of fungal mitochondria with those of the highly conserved regions in retroviral polymerase genes (Fungal mitochondrial introns are divided into two classes based on the presence of one of two sets of short sequence blocks that are

thought to determine their secondary structure in RNA^{16,17}). These regions are conserved between the *pol* genes of all known vertebrate retroviruses and retrovirus-related elements in yeast, plants and insects^{18,19}. The new work shows that each of the homologous segments that unite these introns are related to a corresponding conserved segment of the retroviral *pol* genes. The order of segments is conserved with a few differences in gap size between them. Lastly, the ten diagnostic predicted amino acids, which are virtually all present in *pol* genes, are similarly conserved in the class II open reading frames. It is thus likely that these open reading frames encode proteins that are related to reverse transcriptase.

What is known about the gene products of these class II open reading frames? The answer is quite a lot and none of it points to their encoding reverse transcriptase. The a1 intron of the *oxi-3* gene (one of the four open reading frames) encodes a 'maturase', which has been elegantly demonstrated by Slonimski and coworkers to be necessary *in vivo* for the splicing out of the intron that encodes it²⁰.

It seems unlikely that a DNA reverse transcript of an intron participates in the removal of that intron from a precursor mRNA; is it more likely that the open reading frame gene product possesses both reverse transcriptase and maturase activity? There is some evidence to suggest that the maturase activity is not critical to the splicing event. Class I and class II introns are probably all spliced by a mechanism closely related to that of the self-splicing ribosomal RNA introns of *Tetrahymena*^{21,22}. It is thought that maturases aid the alignment of precursor RNAs, which then splice auto-catalytically^{6,21,22}.

Other nuclear genes have been identified in yeast that are necessary for splicing of mitochondrial introns in this fungus. A dominant mutation (probably a mis-sense point mutation) in one of these, *NAM-2*, cures a splicing deficiency resulting from the inactivation of the b4 maturase (which is required for splicing of the b4 intron of the *cob* gene containing it and also of intron a4 of the *oxi-3* gene²³). The *NAM-2* mutant gene requires a gene product of the region containing exon 4 and intron a4 for this action. These data show that maturases participate in splicing at some stage, but under some conditions a particular maturase is dispensable and there is an independent requirement for other gene product(s). They do not show what enzymic activity maturases possess. To complicate the picture further, if the wild-type *nam* allele is totally inactivated, the mitochondrial genome is annihilated,

forming cytoplasmic *petite* mutants²³.

Michel and Lang argue that maturase function may be an accessory activity, regulating the level of the intron gene product, the major function of which is the maintenance of such introns by dissemination through the mitochondrial genome. Seen in this way, the fungal mitochondrial genome is an environment being exploited by an autoregulated class of mobile genetic elements. This is perhaps taking speculation a bit too far on the sole basis of amino-acid homologies. What is obviously required is the demonstration of reverse transcriptase activity in yeast mitochondria and, one hopes, from *in vitro* constructs containing these class II maturase genes. It will also be important to demonstrate transposition of class II introns, if possible. The authors cite two pieces of circumstantial evidence implying the presence of reverse transcriptase activity in fungal mitochondria — the occasional perfect excision of some *Saccharomyces cerevisiae* introns (presumably by gene conversions by a complementary DNA copy of the spliced mRNA), and the existence of a mitochondrial plasmid in *Podospora anserina* that is identical to one of the four introns they present. These arguments are persuasive but not conclusive.

If the authors are correct, these are the first identified reverse transcriptase genes of cellular rather than viral origin. It has been proposed that retroviruses evolved from mobile DNA elements that picked up an ancestral reverse transcriptase gene while wandering around the genome^{24,25}. A cornerstone of this theory is the cellular reverse transcriptase gene, which has been conspicuous by its absence from the literature till now. Perhaps some maturases are the progenitors of modern reverse transcriptases.

Are all introns explicable as mobile, self-splicing elements? Probably not; the class I and II introns are rare except in fungal mitochondria, an exception being the *Tetrahymena* 26S rRNA intron. Other introns fall into two classes, the nuclear mRNA introns and the transfer RNA introns. Neither of these possesses genes that affect their transposition, although it is always possible to invoke a small number of yet uncloned trans-acting genes. Both classes furthermore are excised by mechanisms very different from the class I and II introns, although mRNA splicing does create circular lariat intron excision intermediates that are reminiscent of the perfect circles created from class I/II excised introns^{3,4}. Nevertheless, the possibility that introns are relics of ancient mobile elements must still be considered. By this hypothesis the large majority of introns have long since evolved away from a migrating lifestyle.

Another possibility is that the class II maturase-containing introns are the descendants of the fortuitous incorporation of a reverse transcriptase gene by genomic rearrangement. The resulting construct

* I am grateful to Donal Hickey for communicating to me his ideas on intron evolution, in particular the possibility that introns are descendants of highly specialized retrotransposons. This will in part be presented as a letter in *Scientific Correspondence*.

would be able to ensure its survival by replicative transposition. This seems a more likely possibility: we know of many examples of DNA rearrangements and the success of mobile elements in eukaryotic genomes is similarly obvious. Of course, if the incorporated reverse transcriptase gene came from a retrovirus we are back at square one in our search for the ancestral gene.

How might an intron transpose using reverse transcriptase? For the intron to survive more than one cycle of retrotransposition, the ends of the element would have to correspond exactly to the region spliced out of the pre-mRNA (this is especially important for intron insertion in open reading frames). The splicing signals would need to be contained in the intron; this is the case for class I/II introns. The most attractive model employs the circular RNA molecules generated from introns after excision. It is easy to imagine reverse transcriptase converting these into a double-stranded DNA circle that would presumably be cleaved where the ends of the intron meet, and inserted into the acceptor site. One problem with this model is that the circles lose a few nucleotides from their 5' ends during circularization²⁶, and these would need to be restored upon integration to preserve the intron intact. One attraction of this model is that DNA circles are used in retrovirus proviral integration, thus to some extent unifying the two mechanisms.

Is there any evolutionary evidence for intron insertion in characterized gene families? The mammalian serine proteases have been thoroughly studied in this respect²⁷. There is strong circumstantial evidence supporting the insertion of introns into the chymotrypsin, elastase, urokinase and human tissue plasminogen activator genes. But these are nuclear mRNA introns and the reservations mentioned that these are potential mobile self-splicing elements must be considered.

One well-characterized example of in-

tron insertion occurs in yeast mitochondria. The class I r1 intron of the 21S rRNA gene of *S. cerevisiae* mitochondria is optional²⁸ and several other yeast mitochondrial introns are similarly heterogeneous. As alleles containing the r1 intron (ω^+ alleles) yield almost entirely ω^+ progeny when crossed with ω^- stocks, the intron seems to invade the ω^- 21S gene²⁹. The mechanism is thought to be a specialized form of gene conversion, with similarities to the yeast mating-type cassette switching at the *MAT* locus. Evidence for this comes from co-conversion of flanking genetic markers during $\omega^- \rightarrow \omega^+$ conversion and the presence of a double-stranded DNA break at the recipient site that is highly reminiscent of the y-z endo cut at the *MAT* locus³⁰. The exciting aspect of this mechanism is that the open reading

frame of the r1 intron is required for this process^{14,15}, showing that the intron encodes a gene product that catalyses its transposition. The r1 open reading frame shares homology with the maturase of the b2 intron of the yeast mitochondrial *cob* gene but not with the class II reverse transcriptase-related introns of Michel and Lang. However, unlike b2, the r1 gene product is not necessary for the splicing out of its own intron.

The connection between either the r1 type of intron or the class II intron of Michel and Lang and the maturase function is still unclear. Further study will not only clarify this issue but may tell us much about intron evolution. □

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Polymer biochemistry

Radical breakdown of lignin

from Alistair Paterson and Knut Lundquist

ALTHOUGH certain fungi have no problem in degrading lignin, one of the major constituents of wood, biochemists have had a problem in explaining the mechanism by which a polymer with so many different inter-monomeric linkages is degraded. Intensive research has culminated in the discovery that the mechanism involves an enzymatic one-electron oxidation of lignin yielding aromatic radical cations that initiate cleavage of the side chains¹⁻³.

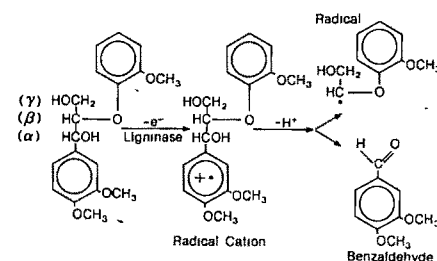
Lignin is synthesized by the addition of phenoxyl radicals, generated by dehydrogenation of the precursor *p*-hydroxycinnamyl alcohols, to the growing polymer chain. Most of the links in the resulting polymer are ether bonds between the central propanoid carbon (C β) of one monomer and the phenolic group of the next, referred to as β -aryl ether linkages, although nearly a dozen other bonds of carbon-carbon and ether type have been reported, giving lignin an irregular structure.

The most effective degraders of lignin are the white-rot fungi, of which the basidiomycete *Phanerochaete chrysosporium* is the best characterized. When growing in mycelial form in static liquid and solid culture, nutrient exhaustion initiates secondary metabolic or idiophasic activities in this fungus. At that time, it begins

to secrete small quantities of 'ligninase', a glycosylated haemoprotein, and to decompose lignin into CO₂. The ligninase was first described in 1983 by Ming Tien and T. Kent Kirk⁴ at the US Department of Agriculture Forest Products Laboratory in Madison, Wisconsin and by Michael Gold's group at Oregon Graduate Centre, Beaverton⁵. Initially, it was reported to require both hydrogen peroxide and molecular oxygen for activity and to degrade both a range of lignin model compounds and extracted native lignin.

The precise mechanism of ligninase activity has since been under detailed study, though limited by the small quantities of purified enzyme available. Now, Kirk and his group¹ and John Palmer and colleagues at Imperial College, London^{2,3} have shown that the initial reaction in the attack on lignin models is a one-electron oxidation that generates relatively stable aromatic radical cations in the lignin polymer. The haem-containing ligninase first reacts with hydrogen peroxide, producing a change in the ultraviolet absorption spectrum of the enzyme akin to that when an oxy-ferryl complex is formed in peroxidases. The enzyme is then able to oxidize lignin models in the presence or absence of oxygen; electron spin resonance studies of the oxidation of dimethoxybenzenes¹ and veratryl alcohol by ligninase yield spectra with signals that are consistent with the generation of the aromatic radicals.

This oxidation can be mimicked by either of two metal-centred oxidant and dimeric lignin model substrates containing an aryl ether linkage. One is tetraphenylporphyrinatoiron(III) in the presence of *tert*-butylhydroperoxide⁶, which is thought to model the oxy-ferryl complex of the monooxygenase cytochrome P-450



Suggested route of lignin depolymerization for a model compound of the β -aryl ether type.

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and peroxidases; the other is tris (phenanthroline) iron(III)³. The results suggest that the central feature of the 'ligninase' mechanism is the creation of the oxyferryl centre of high redox potential. Study of enzymatic attack on the lignin model substrate shows that a radical cation is formed by removal of an electron from the aromatic ring. The model compound then undergoes α - β alkyl cleavage with formation of a benzaldehyde and a radical, as shown in the figure. The latter can react with molecular oxygen, explaining the oxygen-18 labelled products observed in earlier reports.

Ligninase is clearly distinct from the metalloenzymes laccase and peroxidase, whose activity towards lignin model compounds has been thoroughly studied. Laccase is a copper-containing phenol oxidase that is produced by ligninolytic fungi but is also involved in the morphogenesis of non-ligninolytic basidiomycetes. This enzyme oxidizes substrates containing free phenolic hydroxyl groups, whereas ligninase is capable of oxidizing substrates with alkoxyl substituents on the ring; in the lignin polymer most of what were phenolic hydroxyls have been converted to the structural ether linkages. The oxidative changes in phenolic substrates — catalysed by ligninase or laccase — differ considerably from those produced in non-phenolic substrates by ligninase^{7,8}.

For some time, experimental evidence suggested that hydroxyl radicals, which are non-specific oxidants, mediate fungal degradation of lignin. But when Fenton's reagent, which chemically generates such radical species, is used to degrade lignin model compounds, the products differ from those of the enzymatic oxidation⁹. The specificity of the enzymatic reaction can be explained if its postulated oxyferryl centre has a lower redox potential than the hydroxyl radical but a higher potential than other peroxidases.

Once generated by the ligninase, the radical cations are themselves able to act as one-electron oxidants which can oxidize sites remote from the enzyme. Thus, despite the attention still being paid to extracellular proteins from ligninolytic *Phanerochaete chrysosporium*, there would seem to be no reason why ligninase (which is capable of cleaving many of the lignin intermonomeric linkages¹⁰) and laccase, together with the organism's oxidative cellulases (which have roles in reducing quinones¹¹, produced by oxidative lignin degradation and in generating the hydrogen peroxide¹² that is required to form the oxyferryl centres)

should not be able to catalyse all the lignin depolymerizations that have been observed up to now. □

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Astrophysics

New detectors for gravity waves

from James Hough

DIRECT detection of gravitational radiation remains one of the most challenging and difficult problems in experimental physics. Success in this field will be of considerable significance both for astronomy where new information about violent interactions in the universe may be obtained, and for physics where fundamental aspects of relativity theories may be checked. Indirect evidence for the existence of gravitational radiation has been provided by observations on the binary pulsar PSR 1913+16 and this provides added encouragement for the development of experiments in the field.

The detection of gravitational radiation relies on sensing the fractional strain or amplitude in space induced by the wave, through the effect on test masses, which may be essentially free or which may be coupled together mechanically. The resulting effective displacements of the test masses increase with their distance apart. Alternatively, the effect of the radiation on the phase of electromagnetic waves may be used. Radiation from possible sources ranges in frequency from 10^{-4} Hz for large black hole interactions and 10^{-3} Hz for fast binary stars to 10^3 Hz for stellar collapses (see table). To some extent the

frequency being searched determines the choice of detection method.

Detectors based on Earth look very promising for frequencies above several tens of Hertz, but are limited at low frequencies by the effects of seismic and man-made noise. At very low frequencies, one cycle per year or lower, pulsar timing is providing interesting limits on the gravitational wave background^{1,2}. In this case the pulsar is acting as a very stable clock, and the effect of gravitational radiation on the phase of its radio signals is being sought. A stable clock on Earth that forms the bases of a spacecraft tracking system can be used for higher frequencies, and Doppler tracking of interplanetary spacecraft has already allowed searches in the millihertz range to be made at a gravitational-wave amplitude level of roughly 3×10^{-14} (ref. 3). The performance is limited by plasma scintillation affecting the radio signals. The significance of this may be reduced in future by using dual tracking frequencies, but better performance could be obtained by using optical frequency tracking. To avoid the effect of the Earth's atmosphere, however, the whole system would then need to be spaceborne.

In the long-term, the performance of

Some possible sources of gravitational radiation

Impulsive sources				
	Amplitude	Predominant frequencies	Rate	Detection method
Black hole events in galactic nuclei and quasars	$\sim 10^{-19} - 10^{-16}$	$10^{-1} - 10^{-4}$ Hz	< 50 per year	Doppler tracking initially then laser interferometer in space
Stellar collapse (at distance of Virgo Cluster)	$\sim 10^{-22} - 10^{-21}$	< 10^3 Hz	~ 1 per month	Cooled bar detectors, laser interferometers on Earth
Neutron star binary decay (out to 100 MPC, for example)	$\sim 10^{-22} - 10^{-21}$	$\sim 10 - 10^3$ Hz	$\sim$ few per year	Laser interferometers on Earth
Continuous sources				
	Frequency	Amplitude	Detection method (with integration for $\sim 10^6 - 10^7$ s)	
Pulsars				
For example, the Crab Nebula	60 Hz	< 10^{-25}	Laser interferometers on Earth	
Binary stars				
Iota Bootes	8.6×10^{-5} Hz	$\sim 5 \times 10^{-21}$	Laser interferometer in space	
Am CVn	2×10^{-3} Hz	$\sim 10^{-21}$	Laser interferometer in space	
Gemina ⁵	3×10^{-2} Hz	$\sim 10^{-19}$	Laser interferometer in space; 'Skyhook' detector	

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laser interferometry between test masses in satellites spaced a million kilometres or more apart looks very promising, with a potential sensitivity⁴ of about 10^{-21} at frequencies below 10^{-1} Hz. In the shorter term, Braginsky and Thorne⁵ propose an interesting detector of intermediate sensitivity (see page 610 of this issue). Their proposed 'skyhook' detector would consist of two masses on each end of a long thin cable with a spring at the centre, the whole system being arranged to orbit the Earth. By sensing the motion of the test masses as transmitted to the spring, a search for gravitational waves could be carried out with an amplitude sensitivity of 3×10^{-17} at frequencies of 10^{-1} – 10^{-2} Hz. This is clearly an experiment to be considered in the near future as it could be ideal for searching for the radiation from a black hole-white dwarf binary system, such as Geminga may be, from which an amplitude signal of approximately 10^{-19} at the Earth at approximately 3×10^{-2} Hz has recently been predicted⁶. Integration of the signal over several months could allow successful detection.

While space experiments are exciting for the future, the potential of ground-based experiments operating over a frequency range from several-tens of Hertz to a few kilohertz seems considerable. These experiments are aimed at the detection of gravitational waves from sources such as stellar collapses, coalescing binary stars and fast pulsars. Astrophysical models are somewhat uncertain, but theoretical predictions suggest that a strain sensitivity of 10^{-21} – 10^{-22} over a kilohertz bandwidth should be adequate for the detection of fast-pulsed sources, and 10^{-27} over a narrow bandwidth would be an interesting level for pulsars.

Current experiments use two main approaches. One, the development of the Weber bar detectors using low temperature techniques to reduce thermal noise, is under investigation at a number of places including Stanford University, the University of Louisiana, the University of Western Australia, and at CERN where the University of Rome are mounting a significant effort. The Stanford detector is already achieving a strain sensitivity of approximately 10^{-18} for millisecond pulses with plans to improve this sensitivity by two orders of magnitude over the next five years, and to design a detector system to cover a wide frequency band (a fuller discussion can be found in ref. 7).

The other approach is to increase the signal size by using freely suspended test masses placed a long distance apart. Such an arrangement is inherently wideband. To avoid absolute length measurements, the test masses can be suspended to give two perpendicular baselines in which the gravitational wave signal will induce a differential displacement. The relative length of the two arms can be monitored by laser interferometry, and the optical phase changes can be enhanced by reflect-

ing the light back and forward between a system of mirrors on the test masses, which may form an optical delay line⁸ or resonant Fabry-Perot cavity⁹. A number of prototypes of such detectors are under development. Two of these use optical delay lines (at the Max Planck Institute, Garching, where the arm length is 30 m, and at the Massachusetts Institute of Technology with an arm length of 1 m), and two use Fabry-Perot cavities (University of Glasgow and California Institute of Technology with arm lengths of 10 m and 40 m respectively).

The performance of these prototype detectors is encouraging: strain sensitivity spectral densities of better than 5×10^{-19} Hz^{-1/2} have been achieved by those with longer baselines. And it is generally agreed that they have reached a stage where the construction of detectors with much longer baselines should be considered. The optimum arm length for a detector of this type is a compromise between possible sensitivity, range and flexibility of experiments, and the relative importance of anticipated noise sources. The availability of large areas of flat land and the cost and timescale of building the apparatus also influences the choice. At present it seems that the optimum arm length is a few kilometres. A detector with its masses fitted with mirrors of very low loss, as have been developed for laser gyroscopes, and illuminated by several tens of watts of continuous laser light from an array of high power argon lasers (or a YAG laser), could achieve a sensitivity of close to 10^{-22} for millisecond pulses of gravitational

waves and equally encouraging sensitivity figures for other types of sources.

To set up a useful 'telescope' array on Earth, it would be best to have at least four such detectors. Currently there are plans to have two in the United States developed from the Caltech and MIT prototypes, one in Germany as an extension of the current Max Planck experiments, and one in Scotland as a development of the work at the University of Glasgow. In addition, there is a possibility of a fifth instrument being built in France. If the construction of these detectors proceeds there is a very real chance that gravitational radiation from astrophysical objects will be directly detected within a decade. Towards the end of this time certain of the spaceborne experiments could be giving results in the low frequency part of the spectrum. Evaluation of results from the experiments on Earth and in space can be expected to herald a new and very exciting era for astronomy. □

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Population dynamics

Water fleas as population model

from John H. Lawton

THE most widely used and familiar equation in population dynamics is the logistic¹. It is also the most boring, permitting as it does just one sort of dynamic behaviour — sigmoid growth to a stable equilibrium from low population numbers and monotonic decline to the same equilibrium from above. Many real populations do more interesting things, as Murdoch and McCauley², using *Daphnia* (water flea) population dynamics, demonstrate on page 628 of this issue.

Some of the complexities of real population dynamics can be mimicked by modifying the logistic to incorporate a time-lag in the density-dependent term¹.

$$dN/dt = rN[1 - N(t-T)/K] \quad (1)$$

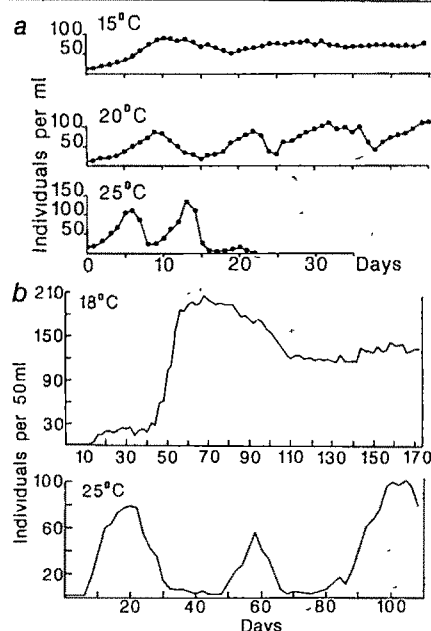
Where N is population density at time t , r is population rate of increase, K is the equilibrium population size, and T is a time-lag in the response of the population to density. When $T=0$ we recover the logistic. With $T>0$ the modified model generates a range of behaviour¹ including sigmoid growth (for $0 < rT < e^{-1}$) or

damped oscillations ($e^{-1} < rT < \pi/2$) to a stable equilibrium K set by food supply or space; alternatively, stable-limit cycles are generated when $rT > \pi/2$. Density-independent disturbances cause non-cyclic fluctuations round the stable equilibrium, and may sufficiently perturb the damped oscillations to yield 'quasi-cycles'³ — fuzzy, but regular, oscillations.

These more complex behaviours recall those discussed by Murdoch and McCauley². Indeed equation (1) has been invoked to explain the wide range of dynamic behaviour shown by laboratory populations of both *Daphnia* and planktonic rotifers, by postulating changes in the relative values of r and T under different temperature regimes^{1,4} (see figure). To date, analogous ranges of behaviour for field populations have been sought among many biologically disparate taxa and food webs (for example ref. 5). But they have not been demonstrated in the field for one species or closely related set of species under structurally similar conditions —

that is, when essential links in the web of community interactions between prey, predators and competitors remain unchanged. Yet the model explicitly predicts different dynamic behaviour under structurally identical conditions, providing only that key parameter values change in relative magnitude.

Time-lags are inevitable in many population interactions involving real organisms. For example, it takes at least a gestation period for animals to respond to increasing food supplies. But although equation (1) crudely incorporates time-lags, it is still a gross simplification of reality. A step nearer to the truth is a pair of equations — one for the consumer pop-



Laboratory populations of (a) the rotifer *Brachionus calyciflorus* and (b) *Daphnia magna* under different temperature regimes but otherwise identical culture conditions. Increasing temperatures change the dynamic behaviour from approximately sigmoid growth or damped oscillations to regular cycles (a modified from ref. 4; b after D.M. Pratt in ref. 10).

ulation (herbivores) and one for the food (green plants), that is, equations in the form of a Lotka-Volterra 'predator-prey' model⁶. Appropriate choice of parameters in such a model again generates the full range of behaviours shown by equation (1) in both prey and predator populations.

Not unreasonably, many field biologists remain sceptical or even hostile^{7,8} to such simple models. They may, after all, appear to do all the right things for entirely the wrong reasons. Therefore, the models need testing. The first step is to find a set of field populations that do some, if not all of the 'right' things. As Murdoch and McCauley show, *Daphnia* and their algal prey seem to be ideal subjects to test the pair of equations, providing that obvious pitfalls, such as fish predation, are avoided. What we then seem to have is the two-species field equivalent of a Lotka-Volterra predator-prey model.

Murdoch and McCauley's *Daphnia*-

algal data challenge the theoretical models in at least three ways. First, and most crudely, do both the relative values of time-lags in the system and *Daphnia* rates of increase agree with the simple predictions of equation (1) for the three classes of populations recognized by Murdoch and McCauley. These are stable (class 1) and cyclic (class 2 and 3) *Daphnia* populations? Second, is it possible to obtain estimates for the parameters of two-species Lotka-Volterra equations, and thus test this more realistic model? For example, is stability (class 1) imposed by mutual interference (intraspecific competition) amongst the *Daphnia*, or by low grazing rates⁶; and do populations in class 2 show quasi- or stable-limit cycles under conditions predicted by the models? Third, as Murdoch and McCauley point out, how is it that class 3 *Daphnia* populations cycle, but not their algal prey? This particular behaviour is not predicted by Lotka-Volterra equations, so either the model or the data are misleading.

It is not difficult to imagine many complexities hidden in these data that may invalidate detailed comparisons with simple models. Examples range from competition between algal species (or between *Daphnia* and other zooplankton), to unsuspected invertebrate predation on *Daphnia* by *Chaoborus* (phantom midges), *Leptodora* (predatory relatives

of *Daphnia*) or water mites, to algal parasites⁹. Indeed, it is extremely unlikely that in the long run, simple Lotka-Volterra models will prove sufficient to model all the complexities of *Daphnia*-algal populations. That is not the function of simple models. Rather, they serve to chart a difficult course between gross simplicity (the logistic) and too much complexity (the field). The models highlight processes that seem fundamental to the dynamics, and signpost the next round of field observations and experiments. In short, *Daphnia* and algae present ecologists with an opportunity to test some important, general, widely used but often maligned models, but at the same time, keeping both feet firmly planted in the muddy complexities of the nearest pond. □

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Glaciology

Contrasts in Vostok core — changes in climate or ice volume?

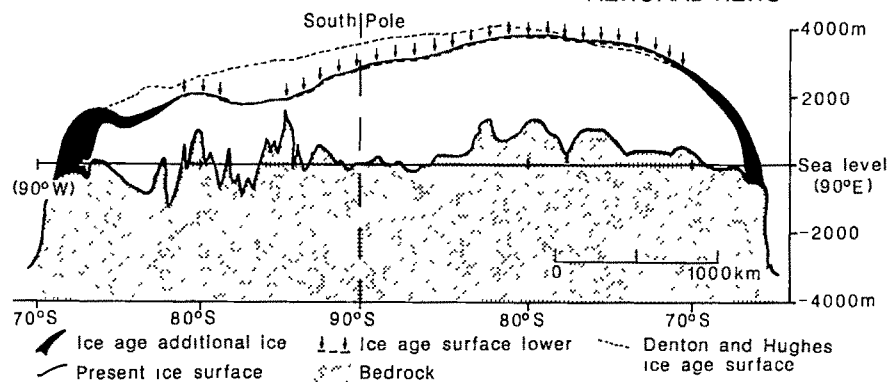
from G. de Q. Robin

CORES from the major ice sheets provide fossil samples of the Earth's atmosphere and yield information on past climates and the mechanisms of glaciation. But obtaining such cores has never been easy, and a really long record has proved elusive. Now Soviet engineers have drilled an ice core of high quality to 2,083-m depth at Vostok station at 78°S, the coldest inhabited place on Earth. This core from East Antarctica is the first to span the last interglacial and recover ice from the preceding glacial period. Papers on p. 591 and 616 of this issue provide an exciting glimpse of results to come from geochemical studies of this 150,000-year climatic record^{1,2}.

Dating of the ice core is based on my observation that current accumulation rates in central Antarctica vary with mean atmospheric temperature above the ground inversion³. These temperatures are reflected in the $\delta^{18}\text{O}$ profile determined by Lorius *et al.*¹ and are used by these authors to extrapolate from present to past accumulation rates. The agreement of the resultant date for the last interglacial with that obtained by other methods suggests the method is satisfactory, as

does the agreement with accumulation rates deduced from the ^{10}Be studies of Yiou *et al.*² Some corrections to allow for increased atmospheric transport of dust⁴ and thus of water vapour during the last glacial maximum may be desirable. The new method is an improvement on dating of marine sediment cores, where uniform accumulation rates between horizons marking prominent climatic boundaries 20 – 60 kyr apart are usually assumed.

Since the annual accumulation around Vostok is less than the surface roughness due to sastrugi (irregularities caused by the scouring action of windborne ice particles), deposition noise levels⁵ in the $\delta^{18}\text{O}$ record are high. The sampling length of 1.5 – 2 m (equivalent to 100 – 200 years) reduces this noise to around $\pm 0.5^\circ$ per thousand (standard deviation). This is similar to deposition noise levels found by comparing measurements from 20 shallow bore holes 600 km away at Dome C station⁶. Separate measurements at 25-m intervals along the whole core (Fig. 1 in Ref. 1) lie mostly within two standard deviations of the continuous curve below 1,406 m, although a difference of four



Suggested zones where the Antarctic ice sheet was thicker or thinner (that is, the ice surface was higher or lower) during the last glacial maximum than at present⁸. This is compared with corresponding surface elevation estimates from Denton & Hughes¹⁶. Thicker ice in coastal regions is due to increased grounding of ice as sea level falls during an ice age. Lower ice surfaces inland show responses to lower accumulation rates suggesting that surface levels of ice streams and glaciers discharging ice from central regions undergo little change or even fall slightly¹⁰.

standard deviations occurs at the interglacial maximum. Within these limits, most fluctuations in the $\delta^{18}\text{O}$ profile can be related to global climatic factors⁷. A notable exception is isotope Stage F; the Vostok record shows much colder conditions than do other marine, ice and terrestrial data for that time.

A rough periodicity of around 40 kyr in the $\delta^{18}\text{O}$ profile, in phase with summer insolation changes at 80°S, strongly suggests a connection with the 'Milankovitch' orbital variations. Together with the similarity in isotope composition at the four minima recorded, this led Lorius *et al.*¹ to conclude that the $\delta^{18}\text{O}$ profile at Vostok primarily records climatic (regional temperature) changes and that effects of changes in surface elevation are small. This relative emphasis on a regional rather than a global explanation of the Vostok climatic record is due in part to the difficulty of finding a satisfactory mechanism of heat transfer between the hemispheres. However, probable changes in the latitude of the effective meteorological equator, in response to changes of radiation balance over the Earth's surface during ice ages, provide an equivalent mechanism (Radok, U. & Riehl, H. personal communication). A global response therefore remains plausible.

The alternative possibility, that ice surface levels over central Antarctica were lower (perhaps by 200–300 m), is supported by recent studies⁸ of factors affecting mean sea level — which may have been 5–7 m higher during the last interglacial. Applying the statement that "climate during the last interglacial was extremely similar to the present" (ref. 9) to the Vostok isotope profile leads to a similar conclusion. The difference between present day and interglacial $\delta^{18}\text{O}$ values could then be interpreted as indicating that the surface elevation around Vostok was about 300–350 m lower during the interglacial than now. If extrapolated to most of central Antarctica, the reduced volume of water bound up in the Antarctic ice sheet would be sufficient to explain the higher sea level, even though the climate

and glacier volumes other than in central Antarctica were the same. This hypothesis avoids the difficulty either of having to melt large sections of the Greenland and Antarctica ice sheets or of having the West Antarctic ice sheet collapse with little change of climate, in order to provide the necessary water. The higher sea level at this time also need not be interpreted as an indication of higher temperature⁹.

Evidence from several sources⁸, including Lorius *et al.*⁴ and Drewry¹⁰, suggests that surface levels in central Antarctica were somewhat lower during the last glacial period than at present (see figure). Because the accumulation rate is very low, the response time of ice thickness to climatic change in central Antarctica is probably tens of millennia compared to a few millennia in coastal regions. Consequently the ice thickness at the start of an interglacial will reflect conditions during the previous glacial period. Marine cores and terrestrial evidence indicate the glaciation from around 200–135 kyr was both colder^{11,12} and the colder temperatures were more prolonged¹¹ than that preceding the present interglacial. Hence average surface elevations in central Antarctica could have been 200–300 m lower around 135 kyr BP than during the Holocene. Applying a similar argument to isotope Stage F suggests that ice then may have been up to 200 m higher than at present following an interglacial buildup. Regional climate would then be some two degrees warmer than that deduced from the assumption of constant elevation in central Antarctica. This would reconcile the Vostok $\delta^{18}\text{O}$ values with general climatic records.

A problem with the surface elevation changes suggested above is that they are larger than expected from steady state glaciological theory. This could be explained by combining aerial and temporal variations of ice accumulation rate in Antarctica. Data from Lorius *et al.*¹ suggest an approximate ratio of 100:60 between interglacial and glacial accumulation rates in central Antarctica. This is supported by the findings of Yiou *et al.*² on precipitation rates. With present day accumulation

rates of 2.5 cm of ice per year at 3,500-m elevation around Vostok and around 10 cm of ice per year at about 2,500 m, the corresponding rates during a glacial period would be 1.5 and 6 cm per year respectively. During an interglacial period of say 20,000 years, 200 m more ice would accumulate at Vostok than during an equal time in a glacial period. At 2,500-m elevation the difference could be as much as 800 m. This would reduce an initial 1,000-m elevation difference between two sites to 400 m after 20,000 years of interglacial conditions, if ice flow were unchanged. Since flow is driven by a shear stress proportional to the product of ice thickness and slope, the dominant influence will not be the increased ice thickness but the decreased surface slope. This will decrease flow once an interglacial period starts and will further increase ice thickness. Such feedback could help explain the rather large elevation changes that it is possible to deduce from the isotope date. It could also explain the present relatively low surface slopes on central Antarctica, which have been cited as evidence of a former surge of the ice sheet¹³. The small positive mass balance in the Antarctic ice budget, noted recently¹⁴, would also be consistent with central elevations that are increasing slowly.

The question of whether the results from Vostok should be explained mainly in terms of climatic effects on an ice sheet of constant surface elevation or by ice volume changes in central Antarctica may be resolved¹⁵ when more detailed isotope and total gas volume measurements become available. Whatever the answer, the Vostok core will have been of considerable importance in unravelling the history of the past global climate, ice volume and ocean levels over a full glacial cycle. □

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Gene control

False starts in translational control of gene expression

from Tim Hunt

THE control of eukaryotic gene expression at the stage of translation of messenger RNA into protein has always been a theoretical possibility, but surprisingly few clear examples have been identified. And even in the well-studied cases, detailed molecular explanations are almost wholly lacking. However, a number of cases of selective translation of mRNA are known and interesting clues to the mechanisms that underlie this kind of regulation are beginning to emerge from recent studies.

These studies focus attention on a number of unanswered questions about how the translational machinery operates. For example, it is known that translation is initiated at an AUG codon in the mRNA; but if there is more than one AUG, how does the ribosome know which to use? What determines the frequency with which a particular message is read? When a ribosome has finished translating an open reading frame in a message, can it start up again if a suitable AUG follows in that same mRNA? Can cells keep a mRNA altogether untranslated in some circumstances, but translate it if the circumstances change? All these questions are yielding interesting new answers, thanks mainly to nucleotide sequence analysis of natural mRNAs and the ability to test hypotheses by making alterations to these mRNAs or by constructing synthetic messages using recombinant DNA methods.

Scanning

According to the 'scanning' hypothesis formulated by Marilyn Kozak¹, the eukaryotic ribosome moves down the mRNA from the 5' or upstream end (which is usually capped) until it encounters the first AUG codon, whereupon translation is initiated. In more detail, 40S ribosomal subunits bearing Met-tRNA_i move along the mRNA until the anticodon of the Met-tRNA_i engages with the first AUG, whereupon the 60S ribosomal subunit joins on, the next tRNA is inserted and the first peptide bond forms. Then the (now) 80S ribosome moves down the mRNA, translating it into protein until a termination codon is reached. This causes the protein to be released from the ribosome, and the ribosome to leave the mRNA, presumably by reversing the initiation process and dissociating into its subunits. These processes, which are not very well understood, may in the light of recent findings, prove to be important in the translational control of protein synthesis: for it turns out that the coding

regions of certain mRNAs are preceded by up to four starts and stops.

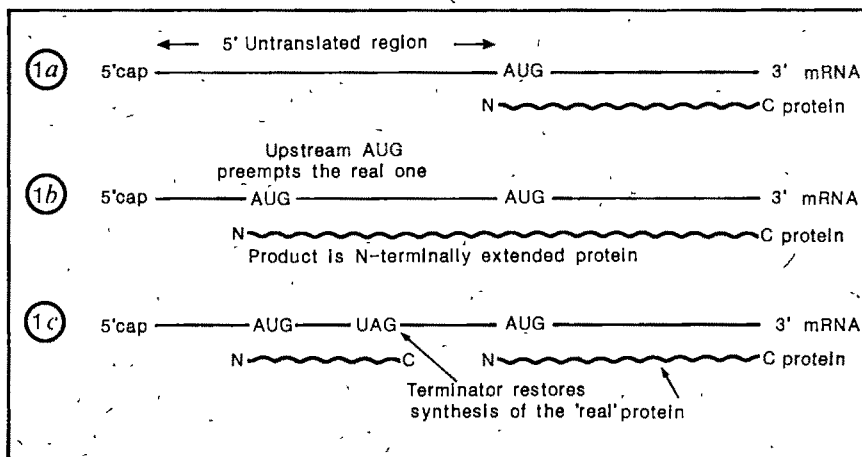
As the Kozak model predicts, the first AUG is used in over 90 per cent of mRNAs whose sequences are known. It usually occurs between 50 and 100 residues from the 5' end of the message, often as the consensus sequence PuN-NAUGG. However, in about 5 per cent of cases, one or more AUGs occur upstream of that which leads to synthesis of the supposed product of the message. What happens when ribosomes encounter these false starts? And, if they are read, what are the consequences?

These questions have been answered in part by deliberately inserting AUGs upstream of the 'real' AUG in mRNAs². In general, it seems that an inserted AUG preempts the natural one, in accord with the scanning hypothesis. If the reading frame of the false start is the same as that

within the first 200 residues of a 2,000 nucleotide mRNA, two AUGs give rise to different proteins formed by alternative starts in the same reading frame, while third protein of completely different length and sequence derives from a AUG in a different reading frame³. A rather similar situation occurs in adenovirus, where an mRNA from the *E1b* gene specifies alternative proteins according to which AUG is used⁴; the first occurs very close to the 5' end of the message and is often ignored in living cells, but it seems to be preferred in cell-free translation systems. These cases do not strictly qualify as examples of translational control, because there is no evidence that the frequency of initiation from the alternative AUGs can be regulated. Rather, they seem to represent cases of economical use of the viral genome, made possible by exploiting wrinkles in Kozak's rules.

Translational control

By contrast, it is now known that expression of the yeast *GCN4* gene (involved in the General Control of Nitrogen metabolism) is regulated primarily at the level of translation^{5,6}. The *GCN4*-encoded protein is a positive transcriptional regulator of many genes involved in amino-acid



of the real one, the result is an amino-terminally extended protein (compare Fig. 1a with 1b). But if, irrespective of the frame of the upstream AUG, termination signals (the codons UAG, UAA or UGA) occur between the false and real starts, ribosomes resume reading the real message, suggesting that they remain attached to the mRNA after terminating synthesis of the first protein, and are able to start up again if a suitable AUG presents itself within a reasonable (but undetermined) distance (see Fig. 1c).

While these experiments support Kozak's rule, there are several cases of different natural AUGs in the same mRNA being read to give alternative products. This suggests that ribosomes can miss an AUG, perhaps if it is embedded in a sequence that is poorly matched to Kozak's consensus sequence.

The mRNA coding for the P and C proteins of Sendai virus is a good example;

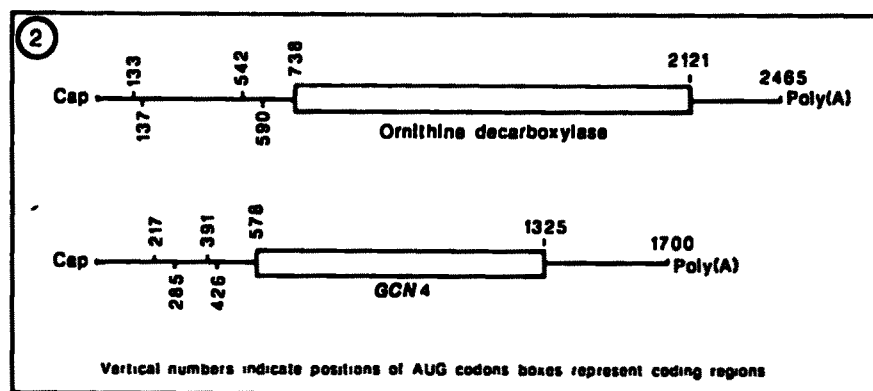
biosynthesis. Its synthesis is boosted something like 100-fold when yeast is starved of any essential amino acid. Its level of expression depends on the activity of a repressor specified by the *GCL* locus, whose activity is in turn somewhat regulated by the *GCN1*, 2 and 3 loci. Until recently it was assumed that this complex regulatory hierarchy operated at the stage of transcription, but it now emerges that the level of *GCN4* mRNA stays essentially constant regardless of the nutritional state of the cell: what changes is the rate at which it is translated. Clues to the mechanism are evident in the structure of the mRNA. It has a 5'-untranslated sequence which, at 5' nucleotides, is much larger than normal and there are four AUG codons upstream of the 'real' one, each followed by a termination codon two or three codons later. A deletion of 280 nucleotides that removes all the upstream AUGs leads to greatly increased translation of the message, consid-

tent with the idea that the upstream AUGs limit translation in some way. Experiments in which point mutations are made in the AUG codons are under way; until the results of these emerge, it will not be clear whether the upstream AUGs are actually read by ribosomes. But, assuming this is the mechanism, how is it triggered by amino-acid starvation?

Amino acid starvation is almost certainly sensed by ribosomes themselves. In bacteria, ribosomes that get stalled on mRNA waiting for a missing aminoacyl-tRNA convert a very significant fraction of intracellular GTP into pppGp and pppGpp. These compounds reach high levels and activate the 'stringent response'. No analogous conversion of low molecular weight phosphorylated compounds has ever been detected in eukaryotic cells despite many searches, so something different must be going on in yeast. The other bacterial paradigm, 'attenuation', acts at the level of transcription and is clearly not directly applicable. Besides, there does not seem to be any correspondence between the amino acids specified by the tiny reading frames following the upstream AUGs and those whose depletion turns on the *GCN4* gene product synthesis. Thus we must await further elucidation of the molecular modes of action of the *GCD1* and the other *GCN* gene products before understanding much more. Probably they are closely involved in the protein synthesis machinery, a hypothesis strengthened by the inviability of *GCD1* mutants.

A strong echo of this story returns in the report of the surprising sequence of a full-length cDNA clone corresponding to ornithine decarboxylase, an enzyme that is involved in polyamine biosynthesis and is extremely rapidly produced by cells that have been stimulated by hormones and growth factors. The interesting thing about its mRNA is that it contains a 737-nucleotide 5'-untranslated region containing four AUG codons upstream of the long open reading frame, followed by terminators 16, 2, 4, and 10 codons later. In broad outline this is highly reminiscent of *GCN4* mRNA (see Fig. 2). The mRNA for ornithine decarboxylase is not very efficiently translated *in vitro*, suggesting that ribosomes rarely reach the proper coding region, but it is not known whether its translational efficiency changes with the physiological state of the cell; if so, does the long leader plays a part in the regulation? It would be very interesting to put this mRNA into yeast and see if amino-acid starvation made any difference to its expression.

There are still relatively few cases in which specific translational control has been shown to occur in eukaryotes, which is one reason the *GCN4* example is so exciting. Several well-known examples, such as the displacement of normal cellular mRNA and the induction of new protein synthesis after heat-shock, or virus-



induced shut-off of host protein synthesis, remain poorly understood at the molecular level. There are several examples of 'masked' mRNA in eggs and oocytes — that is, messages that are very abundant but essentially untranslated until after fertilization. The mRNA that encodes the small subunit of ribonucleotide reductase in clam and sea urchin eggs is a very good example¹. We do not have the complete sequence of any of the mRNAs that show this behaviour, nor any other possible clues as to the mechanism involved. Another rather striking example of developmentally timed 'unmasking' of mRNA is provided by *Xenopus* fibronectin mRNA, present in unfertilized eggs but untranslated until about the time of the midblastula transition, 6–7 hours after fertilization². It would be well worth looking at the 5'-untranslated leader of this message to see if it gives any clues to the masking mechanism. A recent and particularly spectacular example of translational control is that in *Volvox*, where the pattern of protein synthesis changes dramatically in response to light³.

Pointers

Two other helpful points about 5'-untranslated regions have been discussed in recent publications. First, when RNA sequences capable of forming strong secondary structure (inverted repeats rich in GC base pairs) are engineered into the 5'-untranslated sequence of a mRNA, translation of the mRNA is reduced⁴. Second, annealing the untranslated sequence (including the initiation codon) with complementary DNA or RNA completely stops ribosomes finding the start of the mRNA, though once ribosomes start polymerizing amino acids, they are apparently able to unwind or peel off such double-stranded impediments to their progress^{5,6}. Control mechanisms could exploit these features; the 5'-untranslated region of mRNA is the first place to look for possible regulatory sequences.

But neither argument nor experiment has yet settled the role of long 5'-untranslated sequences, with or without upstream AUGs or secondary structure, in the regulation of translation of mRNA. It is certainly not the case that all mRNAs with long leaders are very inefficient; encephalomyocarditis viral RNA which has

an exceptionally long untranslated segment, which includes several upstream AUGs, is one of the most active messages known, both in reticulocyte lysates and infected cells. By way of contrast, the case of *c-myc* mRNA, which normally has a 5'-untranslated region of well over 400 nucleotides, seems to be controversial. It has been suggested that chromosomal rearrangements that cause shortening of this region result in increased expression of the protein by increasing the frequency of translation of the mRNA⁷, but two very similar experiments designed to test this directly (using synthetic mRNAs synthesized *in vitro* from cloned templates by SP6 RNA polymerase) have given conflicting results^{8,9}. An analysis of intact cells, which is anyway much more difficult, has not resolved the conflict¹⁰.

Thus, while it remains easy to imagine the existence of proteins that act as specific translational repressors and enhancers by binding to particular regions of the primary or secondary structure in the 5'-untranslated regions of mRNA, not one authentic example has yet emerged from years of studying mRNA and messenger ribonucleoprotein particles (excluding the poly(A)- and cap-binding proteins, which do not seem to play regulatory roles). I hope yeast will provide the real thing. □

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Photosynthetically active radiation — a new unit

SIR—It is meaningful and useful, and now fashionable in studies involving photosynthesis, to express light intensities as micro-einsteins of photosynthetically active radiation per square metre per second: $\mu\text{E m}^{-2}\text{s}^{-1}$ (PAR). This unit is not a standard international one, as is a mole of photons. It could be confusing since to physicists the Einstein number is a velocity relative to that of light. In fact, it is obviously not a purely physical unit but a physico-botanical one, since spectra of photosynthetically active light differ among green plants, red and brown algae, diatoms, cyanophytes and other photosynthetic bacteria according to their respective complements of photosynthetic pigments: chlorophylls, bilin pigments, and so on. It is awkward to type, since it involves negative exponents and a Greek letter found on few typewriters. It is also a long phrase to use in common parlance, and referring to it briefly and colloquially as a "micro-einstein" is wrong.

I therefore suggest that for physico-botanical usage, in such fields as plant physiology, limnology and oceanography, we use a new nominal unit, the albert: 1 alb = $1 \mu\text{E m}^{-2}\text{s}^{-1}$ (PAR). (This continues to commemorate Albert Einstein.) It is a derived unit, like a joule or a roentgen. Very approximately, one kilo-lux is equivalent to about 20–40 alb, while full sunlight provides a light intensity in the range of 2,000–4,000 alb (depending on the latitude and on the kind of photosynthetic system involved).

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Splicing and the evolution of introns

SIR—The recent suggestion by Cavalier-Smith¹ that introns have evolved by duplicative transposition within eukaryotic genomes is one which we believe deserves serious consideration. We would, however, like to offer an alternative to the model of transposition at the level of DNA. We propose that if introns are indeed the vestiges of transposable elements, they represent a class of retrovirus-like transposons or retrotransposons². The attraction of this latter model is that it gives the splicing mechanism a central role in the evolution of introns. There is a growing body of evidence to suggest that splicing is intron encoded^{3,4}. Consequently, it is reasonable to look for evolutionary explanations of the splicing mechanism in the context of the evolution of the introns themselves.

In our view, the ancestral intron genomes were replicated into RNA copies simply because of their insertion within

transcriptionally active regions of the host genome. Splicing was necessary not only to minimize negative effects on host gene expression⁵ but also, perhaps more importantly, to generate new copies of the intron genome free of flanking exon sequences⁶.

These spliced intron copies were then available for reverse transcription and reinsertion elsewhere in the genome. Most modern introns have probably lost much of their original genetic content and may be considered as degenerate evolutionary relics. An exception is the set of splicing signals which must be retained because of its importance to host cell survival.

Finally, we would like to point out that any particular model of the origin of introns does not argue for or against a subsequent short-term or long-term evolutionary role for these elements within the eukaryotic genome.

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Current density and permittivity

SIR—John Maddox (*Nature* 316, 101; 1985), in his article on Maxwell's displacement current should have been more careful with his electrical units. J is not a current but a current density measured in A m^{-2} . The preferred name for ϵ is permittivity; it is not a scalar but in general a second rank tensor. The name dielectric constant for ϵ should be avoided, as ϵ usually varies with temperature and frequency and can vary with applied field strength. (See *Quantities, Units and Symbols*, Royal Society, London, 1975 and addenda 1981)

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Null hypotheses and the misuse of statistics

SIR—Van Valen pleads for greater freedom for the applied scientist constrained by the "narrow Neyman-Pearson view of statistics as hypothesis-testing" (*Nature* 314, 230; 1985). He advocates a more flexible use of statistics and although in the main we do not disagree with this, our own experience in medical statistics leads us to believe the problem is more fundamental than misuse of null hypotheses.

Statistics involve three ingredients — technical statistics, language and method. Mastery of all three would be ideal. Mastery of technical aspects is a minimum

requirement, based on the organization of ideas according to established recipes defined by previously encountered 'typical research problems. The technical element then is more adapted to provoking action than to reflection, and if we agree that reflection followed by action underlies scientific progress, then we might reasonably consider it perverse for a researcher to be incapable of formulating hypotheses in the absence of stimuli from a computer.

The language of statistics is that of mathematical models and probability theory underlying the calculations. With the advent of computer packages and a much wider access to P -values, increasingly solicited by journals, mastery of the language is rapidly becoming seen as a specialist area only of use in unravelling the most complex situations.

Method is concerned with the fundamental approach and not immediate operational questions. Under this heading we have for example Bayesian techniques, parameter estimation and . . . hypothesis testing. The mistake is to regard these approaches as fundamental principles and to try and squeeze all problems under one or another heading. Worse still is to try and answer questions using one approach which can only be answered using another. To take epidemiology as an example, we almost have to admire the ingenuity whereby, to avoid modelling, problems are formulated in terms of the most untenable null hypotheses. Researchers aware of differential effects and yet with hopelessly inappropriate means for quantifying them find themselves writing $P < 0.0001$ to underline an effect of possibly greater importance than one for which, for instance, $P < 0.01$. Such an expression can find little motivation or meaning in the asymptotic framework of the distribution theory underlying any test, even when appropriate, and yet the optimistic expression $P < 10^{-7}$ is by no means unknown in the medical literature.

To would-be users of statistics it should be made clear that the acceptance or rejection of a null hypothesis does not establish scientific knowledge with the same degree of conviction and this is entirely consistent with Popper's outlook. Unfortunately we are tempted to overlook this fundamental logical asymmetry: thus while some will invoke a non-significant result as evidence for the equivalence of treatments others will invoke a non-significant result as proof of the comparability of patient groups before treatment. In contrast, a significant result will almost always be given a weight disproportionate to its scientific import, the constant of disproportionality varying inversely with the level of significance.

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Between fact and passion

Alan Cottrell

No Immediate Danger: Prognosis for a Radioactive Earth. By Rosalie Bertell.

The Women's Press, 124 Shoreditch High Street, London E1 6JE: 1985.

Pp.435. Hbk £11.95; pbk £5.95.

Power Production: What are the Risks? By J.H. Fremlin.

Alan Hilger: 1985. Pp.318. £22.50, \$33.

How extraordinary it is that two such books as these, written by obviously sincere, intelligent, dedicated and independent authors on the same scientific subject—the risks to the general public of nuclear radiation—should reach such diametrically opposite conclusions. The ground they cover, particularly the biomedical effects of radiation from nuclear power production, is virtually the same, although Dr Bertell's text extends to nuclear weapons production and testing, which she regards as inseparable from nuclear power, and Professor Fremlin's covers in some detail the risks from other methods of energy production, which are dealt with only cursorily and optimistically by Dr Bertell. The difference between the books is all the more extraordinary considering that, for both authors, the preservation of the natural world and its resources is a major objective, as is also the raising of the standard of living in the Third World. Furthermore, both see nuclear warfare as a total catastrophe for everyone. Yet, for Fremlin nuclear power is one of the few bright hopes for mankind on an overcrowded planet, whereas for Bertell it is an ultimate evil, to be attacked with the zeal of a crusader.

Fremlin's is a scientist's book. Cool analysis and numerical estimation are its weapons, used with great skill and elegance to expose many a foolish notion or false argument; for example, that 1 kg of plutonium would be sufficient to kill off all life on Earth. Thus

The natural uranium, radium and polonium in the ocean give off about 40 alpha particles per gram of sea-water per year. One kilogram of plutonium distributed in the oceans of the world . . . would give about 1 alpha particle per gram in 50,000 years [p.263].

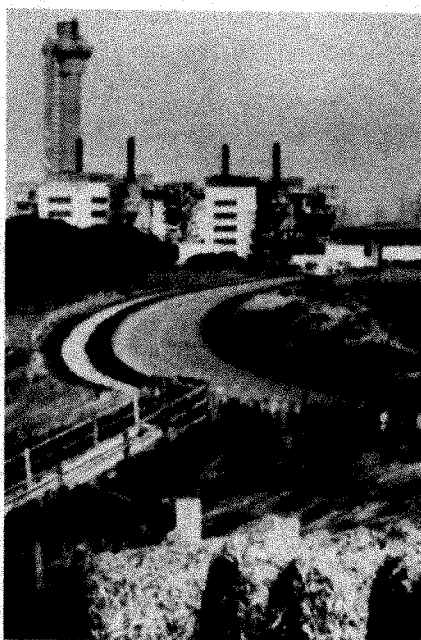
In addition to his opposition to the unnecessary use of fossil fuels, Fremlin's main bias is "in favour of facts" and it is this that has led him to expose and attack false arguments used by anti-nuclear campaigners and disgracefully irresponsible treatments by the media, such as the blatant distortion presented by Yorkshire Television in Britain on 1 November 1983 (p. 275).

Dr Bertell is a scientist but hers is not a scientific book. It is a tirade and manifesto, brilliantly written, but with the set mind of the zealot. Passionate description and harrowing anecdote are her weapons, used with great skill and vividness to re-

mind the reader of what must never be forgotten; of the immense horrors of Hiroshima and Nagasaki. But Bertell treats inconvenient facts more disdainfully than Fremlin. Thus, about the genetic effects of radiation she writes

the data accumulated at Hiroshima and Nagasaki did not give the desired answers. Either through ineptitude or loss of survivors of the bombing . . . the researchers failed to find any severe genetic ills . . . [p.47].

If the facts do not suit the argument, so much the worse for them! The passion also



Power of good? Part of the British Nuclear Fuels Sellafield site in Cumbria, UK.

leads to some surprising assertions. Thus, mankind's nuclear activities have caused acid rain (p.56), the change in the El Niño ocean current (p. 102), and earthquakes (p. 163), as well as 16 million casualties. It is all a conspiracy of militaristic governments, especially Western ones. Even Pearl Harbor was a tactic of the United States designed to strengthen the fighting attitude of American people (p.151).

In a sense, these two books re-enact the classical contest between heart and head in their attempts to find a better future for mankind. Bertell advocates the Utopian future of a global village in which

the fundamental human needs for food, shelter, clothing, medical care, education, work and social dignity are met, so that the tremendous human potential for creativity, art,

science and culture is freed [p.295].

Magnificent, but is it not what mankind has always wanted? And how do we get it and maintain it? Bertell's answer is that

the money for food and celebration of life is being needlessly squandered on weapons and unneeded technological mega-projects [p.296].

Beating swords into ploughshares has been a desirable goal for endless generations and in each new one it has stumbled against the same old problem; human aggressiveness. This is why the heart is not enough. Its solutions are idealistic, planned for angels, not human beings. The head has also to play its part, to seek realism and practicality in the face of human nature as it stands. Peace with goodwill is what Bertell wants, but these may be beyond mankind's reach at present, in which case the grudging peace without goodwill that now exists between the nuclear superpowers may be the best that we can realistically have. But for Bertell, the concept of deterrence is simply not admitted; she is sure that the nuclear powers are preparing to go to war, not trying to dissuade each other from war, in spite of the fact that the European corner of the globe—traditionally a most belligerent region—has enjoyed an unprecedented period of freedom from war throughout the nuclear age.

Fremlin's goals are more limited but more attainable. The people of the world need energy. The big and rapidly multiplying poor in country villages in Africa and Asia need cooking fuel, clean water, practical education and time.

Kerosene and coal are alternatives to the destruction of the forests and the rich countries should be systematically planning to conserve these as effectively as possible and as soon as possible. Compared with the disastrous effects inevitable in an overcrowded world, none of the health hazards of any form of energy production are of any importance at all . . .

The Third World can be helped in this respect

. . . if the richer countries use as much wind and solar energy as they can, develop nuclear energy . . . and use nuclear electricity to replace oil, coal and gas . . . [p.280].

Both books brilliantly expound their author's views but it seems likely that both will fail to persuade unsympathetic readers to change theirs. Fremlin's sophisticated rapiers, needle-sharp though they are, are unlikely to pierce the hermetic seals in which the anti-nuclear protesters have insulated themselves against contamination from uncongenial scientific facts. And the unrelieved damning of all things nuclear by Bertell appears too obsessive to be convincing. To this reader at least, the truth seems less likely to dwell among her passionate advocacies than among Fremlin's cool arithmetic. □

Sir Alan Cottrell is Master of Jesus College, Cambridge CB5 8BL, UK.

Myth, magic and the common solution

Anthony W. Clare

Sherlock Holmes and the Case of Dr Freud. By Michael Shepherd. Tavistock: 1985. Pp.31. Pbk £2.95, \$5.95.

In Nicholas Meyer's *The Seven Per Cent Solution*, Sherlock Holmes, suffering from a severe mental disorder with delusional ideas concerning the machinations of a harmless Professor Moriarty, meets and is treated by Sigmund Freud who is at the time a young physician in Vienna earning a reputation for himself treating the neuroses by an exciting new method of exploration. Cured by the treatment, Holmes joins forces with Freud and together they engage in a successful hunt for a villain who is attempting to precipitate a world war.

Michael Shepherd, Professor of Epidemiological Psychiatry at the Institute of Psychiatry in London, opens what is a deceptively temperate essay with this revisionist view of Conan Doyle's hero. The reason he does so is quite simply that he is about similar business with regard to the sage of Vienna and the founder of psychoanalysis. Sherlock Holmes made the external world coherent by solving mysteries using a method of procedure

which, although appealingly labelled as deductive and logical, was in actual fact intuitive and illogical. However, so attractively human was it that it was and continues to be enormously popular and enjoyable in contrast to the numbing tedium of the real process of detection. Freud, likewise, appeared to make the world comprehensible by solving internal mysteries by a method which, Shepherd argues, was no less intuitive and illogical whatever the claims for scientific status made by Freud and his successors on its behalf. In precisely the sense that Holmes is a caricature of the real life detective, so Freud is a caricature of the psychiatrist.

However, there is more to the link between the fictional private eye from Baker Street and the actual therapeutic ear from the Berggasse. Both Holmes and Freud are the stuff of which heroes are made. Shepherd quotes Brigid Brophy and her view that the cause in which the modern detective employs his method and skill remains the same as that in which the Greek hero used his magic powers — "the deliverance of the population from a threat". In sociological terms, the detective story offers its readers a reassuringly comprehensible and predictable world in which those who try to upset the established order are always detected and punished. Freud's great case-histories read like stories in a Holmesian omnibus and a contemporary novelist, D. M. Thomas, has gone so far as to argue that in his clinical accounts Freud was often fictionalizing, as intent on getting "a good story, a well-shaped classical Greek story, as to get at the truth". Freud and Holmes, Shepherd suggests with more than a touch of mischief, are examples of Nietzsche's *ubermensch*, the new man, detached, quasi-omniscient, a contemporary hero of an ancient legend.

It is an effervescent, provocative, humorous yet profoundly serious argument, and Shepherd elegantly draws on the sympathetic effusions of many of the literary supporters of psychoanalysis to illuminate his view that psychoanalysis, wherever it ultimately belongs, appears more at home within the sphere of myth than of medicine. Others have had the same idea (for example an equivalent argument is mounted in Ernest Gellner's recently published *The Psychoanalytic Movement*), but few have made the case with such an economy of words and breadth of scholarship. To those who know Shepherd and his work, this lively appetizer will maddeningly remind them of the more substantial meal he could cook if the spirit but moved him. Disciples of Freud and psychoanalysis may well breathe a sigh of relief that to date it has failed to do so. □

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The fitful gleam

P. W. Hawkes

Statistical Optics. By Joseph W. Goodman. Wiley: 1985. Pp.550. £49.85, \$59.80.

FLICKERING, glistening, twinkling, shimmering: the literary vocabulary of optics should lead us to expect statistics to play a large part in the subject even if the scientific language is more sober. There are indeed branches of optics — coherence, speckle, some image restoration methods, image compression, some aspects of pattern recognition, weak signal detection — which require a thorough knowledge of statistics, but the first books on probability with special reference to optics have appeared only very recently. The first, *Probability, Statistical Optics, and Data Testing*, written by B. R. Frieden and published by Springer-Verlag, appeared in 1983 and set a very high standard. Although Goodman's new text contains some material that is also to be found in Frieden, the two books are otherwise very different in coverage.

Statistical Optics opens with a brisk account of probability theory and random processes, written in the spirit of a revision aid. Individual chapters are then devoted to first-order statistics, the traditional study of temporal and spatial coherence in terms of second-order statistics, situations in which higher-order coherence arises, image formation with partially coherent light and the phase problem, propagation through random media and, finally, the detection of weak optical signals.

The ratio of optics to statistics is uneven. The chapter on first-order properties is a successful attempt to inculcate statistical thinking habits into students of optics, through the study of thermal light and laser light. The following chapter, on coherence, follows the reasoning made familiar by such standard texts as Born and Wolf's *Principles of Optics* (Pergamon, 1980) and the same is true of most of the later chapter on effects due to partial coherence in imaging systems. The latter concludes with a very brief account of phase retrieval and a slightly longer section on speckle. In the case of phase retrieval, there is hardly any discussion of the extremely important problem of noise in the various reconstruction methods, which is a disappointing and surprising omission because this is an eminently statistical topic. The chapter on higher-order coherence (of which subject it is distinctly less easy to find an elementary account) is much more useful.

The two concluding chapters, on imaging through randomly inhomogeneous media and fundamental limits in photoelectric light detection, are genuine statistical optics, in which the interdependence between the two fields is shown clearly. Goodman leads us through the theory of

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random amplitude and phase screens and then goes on to examine propagation through thick layers of turbulent material, typically the atmosphere. The discussion concludes with an analysis of stellar speckle interferometry. In the final chapter, he presents the semi-classical theory of photodetection, including photo-counting statistics, the notion of degeneracy parameter, and noise limitations in various kinds of interferometry.

The text is clearly and interestingly written, but one serious criticism remains (probably reflecting more on the status of optics in university physics curricula than on Professor Goodman): this is not a complete optics course, so students must presumably attend others in which they could reasonably be expected to learn about partial coherence and its propagation. Such topics could therefore have been covered much more concisely, leaving space to deal thoroughly with material more likely to be new: the phase problem, super-resolution, and the host of statisti-

cal methods and notions used in image processing.

For physicists, then, *Statistical Optics* will be welcome as a guide to the parts of statistics needed in optics, but it does not go deep enough into the various applications for the student who plans to make optics his career, or at least the subject of his PhD research. For the electrical engineering community, however (and Professor Goodman's chair is in this subject), the book probably provides a useful initiation into the optical world for those whose preoccupations bring them into contact with the topics covered but who do not need to go into them too deeply. There is thus still room for a book in which the author assumes that his readers have mastered the statistics in this book, or in Frieden's, and are now ready for a thorough examination of statistical optics. □

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A life in theory

Esther R. Phillips

The Mathematics of Sonya Kovalevskaya. By Roger Cooke. Springer-Verlag:1985. Pp.234. DM96, \$29.80.

SONYA Vasilievna Kovalevskaya (1850–1891) was a mathematician, poet, novelist, dramatist, political tract writer and, some would add, revolutionary. She embraced the nihilism of the 1860s, with its optimistic, perhaps naïve, faith in the power of science and reason to establish a better society, and set out, against great odds, to obtain the university education that would enable her to take part in the building of a new society.

In the course of her turbulent and sometimes melodramatic life, Kovalevskaya contracted a fictitious marriage (1868) which permitted her, along with her sister and friends, to travel abroad to receive the university education denied them in Russia. Barred from attending classes at the University of Berlin (as were all women), she persuaded Karl Weierstrass, one of the leading mathematicians of his time, to give her private lessons covering the material he presented in his university lectures. Her studies were abruptly interrupted in 1871 when she journeyed to be at the side of her sister, an active participant in the short-lived Paris Commune; here Kovalevskaya served as a nurse for five weeks.

Returning to Berlin she resumed her studies, and under Weierstrass's direction she received a doctorate in mathematics (the first awarded to a woman) in 1875. A five-year break from mathematics followed, during which she returned to Russia, had a child, engaged in disastrous real

estate speculations, wrote several non-mathematical works and plunged into a frenetic social life in Petersburg. With the help of Weierstrass and G. Mittag-Leffler (also a former student of Weierstrass) she obtained a position (the first woman to do so) at the University of Stockholm, where, in 1889, she was appointed professor for life. The previous year she had received the Bordin Prize of the Paris Academy of Sciences for her memoir on the rotation of rigid bodies.

Finally, in 1889 she was elected to the Petersburg Academy of Sciences (again, a first for a woman) as corresponding member. At the time of her sudden and untimely death in 1891, Kovalevskaya was at the height of her career — an “international celebrity”, as Roger Cooke describes her (p.116).

In the book Cooke has provided a complete account of Kovalevskaya's work and a sympathetic, but not adulatory, assessment of it. Although numerous articles with biographical details have been published, there is only one full length biography in English (with almost

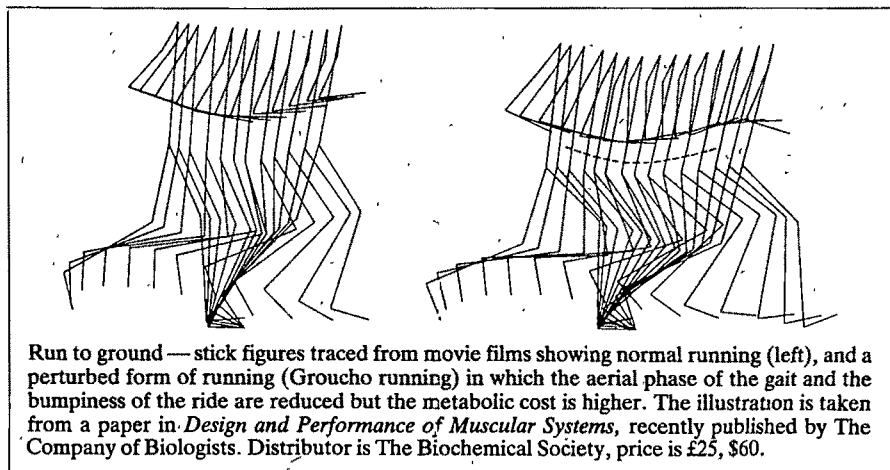
no discussion of her mathematics), A.H. Koblitz's *A Convergence of Lives* (Birkhäuser, 1983).

By describing in some detail the investigations of her predecessors, the author has quite correctly presented Kovalevskaya's achievements in a proper historical context. For example, in discussing one of her best known results — usually referred to as the Cauchy-Kovalevskaya theorem — he distinguishes between the modern textbook version (that it is an existence and uniqueness proof) and what Kovalevskaya, working within the Weierstrassian tradition, had set out to do, namely to demonstrate how analytic functions could be defined by means of formal power series arising as solutions of partial differential equations. Cooke has widely supplemented the description of Kovalevskaya's results with appendices containing technical details and has provided suitable “translations” of the original results into contemporary language. However, despite the author's intention to write a book for non-specialists (p.vii), I do not believe that the more technical sections will be accessible to readers who have not (at the very least) completed a course in complex function theory.

Today Kovalevskaya's achievements have taken on something of a mythical quality, and she has been elevated to a prominent position in the feminist pantheon. Thus a valuable part of this volume contains assessments of her work made by her contemporaries, H. Poincaré, A.A. Markov, V. Volterra, G. Mittag-Leffler and (in 1926) F. Klein. Cooke generally concurs and concludes that she was

a competent, creative mathematician who produced some valuable work and a few works of minor importance, making an occasional mistake in the process... she would not seem quite so remarkable if she had lived a century later, when no one is surprised to see a competent woman mathematician [pp.178–179]. □

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Run to ground — stick figures traced from movie films showing normal running (left), and a perturbed form of running (Groucho running) in which the aerial phase of the gait and the bumpiness of the ride are reduced but the metabolic cost is higher. The illustration is taken from a paper in *Design and Performance of Muscular Systems*, recently published by The Company of Biologists. Distributor is The Biochemical Society, price is £25, \$60.

Burning conditions

D.B. Spalding

The Mathematical Theory of Combustion and Explosions. By Ya. B. Zeldovich, G.I. Barenblatt, V.B. Librovich and G.M. Makhviladze. Consultants Bureau (Plenum): 1985. Pp.597. \$95, £90.25.

IN ESSENCE, the mathematical theory of combustion is simple: molecules interact, and are created or destroyed, as a consequence of the reactions studied by chemical kineticists; these sources and sinks give rise to concentration and temperature gradients which engender diffusional fluxes, of the kind represented by the laws of Fick and Fourier; simultaneously, either because of the density changes or of conditions imposed at the boundaries, the products and reactants are set in motion in a manner governed by the Navier-Stokes equations; the high temperatures give rise to radiative transport of energy in accordance with the classical laws; and the interchanges of mass, momentum and energy with the surroundings can also be expressed quantitatively.

A given combustion phenomenon can therefore be represented by an established set of simultaneous partial differential equations, complicated by the fact that many of the included variables (for example density, concentrations, temperature) are linked by known algebraic relations expressing the nature of the materials in question. Once the initial and boundary conditions have been fully worked out, the problem in question is then ready for solution.

Of course, the non-linearity of the equations, and the multi-material, multi-dimensional character of most combustion phenomena, demand the use of numerical methods and of powerful digital computers. A consequent danger is that the employment of such formidable apparatus will so distract and overburden the theorist that he fails to achieve that perception of interrelationships, and ability to anticipate outcomes, which we call "understanding".

Any combustion specialist who wishes to strengthen his understanding will do well to study the present book. Soviet scientists have done much of the pioneering research on this subject, particularly in the formulation of concepts and provision of explanations of major phenomena. The senior author has been especially prominent (with Semenov, Frank-Kamenetskii, Shchelkin, Vulis and others) while his co-authors too have themselves made notable contributions.

That pioneering work was performed before electronic digital computers were available, for which reason the mathematical formulations had to be simplified; but it was conducted with realism and intelligence, and without extremism. Just

enough of the physics was retained (and especially the steep temperature dependences which give rise to nonlinearity) for the important phenomena of ignition, propagation, instability and extinction to be understood; and simple approximate methods of solution were devised for the resulting equations.

The book retains many of the virtues of the early works. But although it pays some attention to more recent trends and preoccupations, it does not follow them very far. The reader will therefore find little about theories of turbulent combustion; and recent advances in large-scale computer simulations of flames are not mentioned.

The original Russian edition of the book appeared in 1980. Its lucidity has been well preserved in the English translation and it can be read with both pleasure and advantage by combustion scientists in the Western world, several of whom will find that ideas which they have attributed to themselves or their contemporaries were already thoroughly understood a generation ago. The authors have, incidentally, addressed a graceful preface to their Western readers, while their introduction, which is the same as that of the Russian edition, reveals their full awareness that combustion is a truly international science. □

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Fit for survival

John Hearn

Reproduction in Mammals, 2nd Edn. Book 4, Reproductive Fitness. Edited by C.R. Austin and R.V. Short. Cambridge University Press: 1985. Pp.241. Hbk £22.50, \$39.50; pbk £8.95, \$14.95.

THE success or failure of reproduction is crucial to survival or extinction, and thus of immense evolutionary importance. Knowledge of the process is also of great consequence in the management and conservation of species diversity in an ever more crowded world. This will continue to be a central question during the next 50 years, over which the world's human population will double to nearly nine thousand million. Much of the increase will take place in developing countries that now provide the remaining homelands of many exotic species.

Our current understanding of reproductive strategies, species variations, the genetic, behavioural and immunological factors in reproductive fitness, and the influence of senescence, is well presented in this volume, the updated version of Book 4 of the first edition of *Reproduction in*

Mammals (published in 1972 under the title *Reproductive Patterns*). It is virtually a different book: two authors of the original complement remain and there are now seven chapters instead of five. All of the contributions are valuable, but among the newcomers I particularly enjoyed the chapters on genetics (Roger Land), environment (Brian Follett) and reproductive strategies (May and Rubenstein). Revision of the series as a whole continues next year with the appearance of the fifth volume, to be entitled *Manipulating Reproduction*.

While we are rapidly improving our appreciation of general principles, we are still desperately ignorant of precise details of the basic factors that limit survival of individual species. Among mammals alone we have an adequate understanding of fewer than 50 of the 4,000 species, including those on which our agriculture and biomedical sciences are based, and our domestic pets. It is ironic that this knowledge has contributed enormously to the success of our own species in helping us to overcome all natural environmental constraints, but in consequence threatens the survival of most other species. There is a pressing need to define the essential requirements of key species, so that conservation and management policies for the shrinking wildernesses genuinely meet the needs of these species and of others that share the same territory. A hard, quantifiable, scientific approach, at the fundamental level achieved in this book, is critical for the future of animals, including man.

To quote from Roger Short's contribution (Chapter 2, on species differences):

Readers ... will have been struck by the enormous diversity of reproductive mechanisms across mammalian species. About the only common factor is that, somewhere along the line, a spermatozoon meets an egg and so a new individual is formed. Everything else is subject to enormous variability, so that generalisations become impossible. This may act as a deterrent to the faint hearted, who are baffled by complexity; in reality it should be seen as an exciting challenge, encouraging us to probe deeper for the reasons underlying the diversity.

The strength of this book, and of the series of which it is part, is that a bewildering complexity and variability of species, physiological mechanisms and scientific disciplines is made readable, understandable and exciting. The contents are right up to date and point the way to further research development. While the book is aimed primarily at an undergraduate audience, research workers and lecturers will also find it invaluable. As a definitive text in a fast-moving field, I commend it as required reading for those interested in reproductive biology. □

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Charting the decline in British science

from John Irvine, Ben Martin, Tim Peacock and Roy Turner

Recent data on the comparative scientific performance of different nations suggest that the decline in British basic science apparent during the 1970s began to level off at the start of the 1980s. However, rather than heralding the beginning of a revival, the evidence suggests that this is merely a temporary halt to the long-term slide.

It is now almost conventional wisdom that British science is in a state of crisis. Recent leading articles in *Nature*, for example, have had a tone of desperation, with headlines such as "University paymasters discredited — the British government's latest policy document on higher education is a disgrace", "More crisis for UK research", and "Parliament's chance to act". A similar, if more restrained, note of despair has been sounded by the Advisory Board for the Research Councils (ABRC) in its latest report, *Science and Public Expenditure 1985*, which was submitted in June to the Secretary of State for Education and Science. In unambiguous terms, this stated that, "We believe that Parliament, the Government and the country as a whole are complacent about the current financial straits of the dual support system" (for financing research in universities).

In these and other analyses of the situation now confronting British science, most attention has been focused on inadequacies of the funds and other inputs to research. Largely for lack of data, less has been said about the effects of financial constraints on the outputs from science. We attempted to remedy this last year in a study for the ABRC which made use of a range of research output indicators (world shares of publications and citations, and figures on Nobel Prizes and patents), seeking thus to provide systematic information on long-term trends in British science.

The evidence suggested that, during the latter half of the 1970s, Britain's basic science declined appreciably, both in regard to relative output of publications and in their impact on the international research community. It was argued that the government may not be providing sufficient support for research (or at least not concentrating it in the right places) to ensure the successful development of new core technologies vital to the long-term survival of British industry.

The subsequent public interest in the results of the study (see *New Scientist*, 1429, pp.25–29, November 1984) apparently reflects a growing realization that, despite all the limitations of scientific output indicators, it will be necessary to make much greater use of systematic information on research performance in for-

mulating a coherent national research policy if Britain is to ensure that the scientific needs of new technology-based industries are met. Trying to steer publicly funded research using only the mechanism of scientific peer-review (or "the informed prejudices of wise men", as it has been aptly described), while totally ignoring quantitative input and output indicators, is somewhat like trying to run the economy using only the judgements of a committee of senior industrialists.

In what follows, we update the figures on Britain's performance in basic research to the end of 1982, and provide more detailed data on trends in some of the main fields of science. The work involved was supported by a consortium of British learned societies who have set up an informal working party (Ad Hoc Working Group for Research Funding, AGREF) under the chairmanship of Professor J. Lamb (St Andrews University, UK) to examine government support for science.

Measuring performance

Perhaps the most readily accessible data on national research outputs are the publication and citation indicators contained in the National Science Foundation's *Science Literature Indicators Data Base*. This is compiled by CHI Research on behalf of NSF for use in the biennial US *Science Indicators* report, and is based on the *Science Citation Index* produced by the Institute for Scientific Information (ISI). (This database is the source of the material pre-

sented in Tables 1–7, and a copy of it is held at the Science Policy Research Unit, University of Sussex.) The primary dataset covers some 2,000 of the world's leading scientific journals over the decade 1973–82, and contains figures on numbers of publications and citations for more than 190 countries and geographical regions, broken down into eight main scientific fields (for example, physics) and, in the case of the publication data, into approximately 100 subfields (for example, optics).

The use of such bibliometric data to indicate research output in basic science entails a number of assumptions. One is that most new scientific knowledge is eventually encapsulated in the form of research articles in learned journals. If this is the case, it follows that aggregate figures on the number of British publications in specified fields compared with those from other countries should give some indication of Britain's relative scientific output and, more importantly, of trends over time. A second assumption is that citation counts (that is, the number of times papers are subsequently referred to by other scientists) can be used to gauge the impact of research findings on the scientific community.

At the outset, we list three important caveats. First, the ISI data are appreciably biased in favour of English-language journals, which means that the scientific contributions of West Germany, France, Japan and, in particular, the Soviet Union

Table 1 UK world share of publications by field of research, 1973–82

Field	Percentage of total					Av.	Av.	Total %
	1973	1975	1978	1980	1982	ann. % change 1973–80	ann. % change 1980–82	
Biology	9.5	10.7	10.8	10.2	9.9	+1.1%	–1.5%	+4%
Biomedical research	9.4	9.6	8.8	8.4	8.4	–1.5%	0.0%	–10%
Chemistry	7.8	8.3	7.0	6.8	6.9	–1.8%	+0.7%	–12%
Clinical medicine	10.4	10.4	9.5	9.3	9.4	–1.5%	+0.5%	–9%
Earth and space sciences	8.1	9.5	8.9	9.5	8.9	+2.5%	–3.2%	+10%
Engineering and technology	10.7	11.0	9.4	9.5	8.6	–1.6%	–4.7%	–20%
Mathematics	7.4	8.2	7.0	7.0	7.0	–0.8%	0.0%	–5%
Physics	7.7	7.4	6.3	5.9	6.1	–3.3%	+1.7%	–21%
All fields combined	9.2	9.5	8.6	8.3	8.3	–1.4%	–0.1%	–10%

are underestimated. Second, the CHI/NSF trend data are based on an essentially constant set of journals established in 1973; papers published in entirely new journals are not covered, with the result that newly emerging or fast growing areas of science may be under-represented. This disadvantage is, however, offset by the fact that more reliable analyses of trends over time can be undertaken. (Since 1981, CHI Research has in fact compiled an alternative data series based on the 3,000 journals scanned by ISI in that year, but their analysis suggests that this larger database may be more, rather than less, biased in favour of English-language countries.) Third, since CHI Research assigns articles to fields and subfields according to the journals in which they are published, some problems can arise in relation to smaller specialities, in particular those of an interdisciplinary nature where researchers publish in a great variety of journals.

Britain's performance

Table 1 contains data on changes over the decade up to 1982 in Britain's world share of publications broken down by field of research. While there has been a decline of 10 per cent in overall world share (down from 9.2 per cent to 8.3 per cent), the picture is uneven. Physics and engineering have suffered significant decreases of 21 per cent and 20 per cent respectively, but biology has marginally increased its world share by 4 per cent over the 10 years. More encouraging, at least at first sight, is that the downward trend evident since 1975 seems almost to have levelled off in the two years since 1980, with physics, chemistry and clinical medicine apparently registering small increases. We shall return to the question of whether this halt in the downward trend is likely to continue or is merely a temporary phenomenon.

On the impact of British work on the international research community, a rather less optimistic picture emerges from the citation figures in Table 2. It can be seen that the decline which began in the 1970s has continued into the 1980s — the world share of citations for all fields combined has decreased by 15 per cent since 1973, with biomedical research, engineering and physics all recording falls of over 20 per cent. (Figures based on the 1981 journal-set are relatively consistent with these values.) Furthermore, although there had been a slight decrease in the rate of decline from 1.9 per cent per annum in the 1970s to 1.2 per cent in the early 1980s, there is little sign in the citation figures of the levelling off seen in the world share of publications.

When the figures for Britain are set against those for the other main industrialized nations, the implications of the long-term decline in Britain's basic science become all too apparent. Table 3 gives a breakdown by field of percentage changes in national shares of world publications

Table 2 UK world share of citations by field of research, 1973–82

Field	Percentage of total					Av. ann. % change	Av. ann. % change	Total % change
	1973	1975	1978	1980	1982	1973–80	1980–82	1973–82
Biology	11.9	12.5	12.8	12.0	11.4	+0.1%	–2.5%	–4%
Biomedical research	11.7	10.4	9.0	8.5	8.4	–0.6%	–3.9%	–28%
Chemistry	11.2	10.2	11.5	10.3	9.8	–1.1%	–2.4%	–12%
Clinical medicine	12.8	12.8	11.5	11.3	11.5	–1.7%	+0.9%	–11%
Earth and space sciences	9.4	9.9	8.9	9.3	8.9	–0.2%	–2.2%	–5%
Engineering and technology	11.5	12.1	10.9	9.9	9.0	–2.0%	–4.5%	–21%
Mathematics	8.7	9.4	7.3	7.8	7.4	–1.5%	–2.6%	–15%
Physics	7.6	7.8	6.1	6.7	5.9	–1.7%	–6.0%	–22%
All fields combined	11.2	10.9	10.2	9.7	9.5	–1.9%	–1.2%	–15%

Table 3 Percentage changes in world publication share of major industrial countries by field, 1973–82

Field	Canada	France	FRG	Japan	UK	USA	USSR	Rest of world
Biology	+6%	–30%	–10%	+24%	+4%	–5%	–7%	+8%
Biomedical research	–10%	–27%	+7%	+64%	–10%	+5%	–7%	–5%
Chemistry	–17%	–17%	+11%	+23%	–12%	–6%	–11%	+12%
Clinical medicine	+4%	–6%	–13%	+62%	–9%	–2%	–17%	+5%
Earth and space sciences	+5%	–4%	+46%	+4%	+10%	–8%	–9%	+12%
Engineering and technology	–15%	+31%	0%	+45%	–20%	–1%	–31%	+22%
Mathematics	–24%	0%	+15%	+52%	–5%	–19%	+74%	+31%
Physics	–19%	+7%	+28%	+38%	–21%	–9%	–16%	+20%
All fields combined	–8%	–9%	+2%	+40%	–10%	–3%	–15%	+9%

Table 4 Percentage changes in world citation share of major industrial countries by field, 1973–82

Field	Canada	France	FRG	Japan	UK	USA	USSR	Rest of world
Biology	+13%	–2%	+13%	+32%	–4%	–5%	–45%	+6%
Biomedical research	–9%	+10%	+48%	+83%	–28%	–4%	–6%	+3%
Chemistry	–14%	+3%	+4%	+59%	–12%	–9%	–13%	+11%
Clinical medicine	+2%	+17%	+3%	+84%	–11%	–4%	–27%	+6%
Earth and space sciences	–6%	+9%	+66%	+35%	–5%	–3%	–34%	+12%
Engineering and technology	–23%	+8%	–11%	+91%	–21%	+4%	–51%	+30%
Mathematics	–10%	+19%	+3%	+53%	–15%	–13%	+5%	+42%
Physics	–23%	+13%	+35%	+69%	–22%	–15%	–23%	+37%
All fields combined	–7%	+8%	+14%	+65%	–15%	–5%	–27%	+11%

over the period 1973–82. Japan has increased its overall share by no less than 40 per cent and the rest of the world by 9 per cent; the main losers have been Britain (–10 per cent), Canada (–8 per cent), France (–9 per cent) and the Soviet Union (–15 per cent), although in the last two cases there are likely to be appreciable biases in the statistics since French and Soviet scientists may well be preferring to publish more in domestic journals not scanned by ISI.

Such an effect would also give rise to an element of bias (probably rather smaller) in the figures on changes in national shares

of world citations given in Table 4. These suggest that Japanese researchers in all eight fields have markedly increased their impact on the international scientific community, with an overall citation share that has risen by 65 percent during the 10 years to 1982. Similarly, West Germany and the rest of the world increased their overall shares by more than 10 per cent, with France also recording a significant increase (8 per cent). Britain and the Soviet Union have again been the main losers, with declines of 15 per cent and 27 per cent respectively. One interesting feature of the table is that Japan's largest gains in

Table 5 Strengths and weaknesses in British science: share of world publications, 1982

Weaker areas: <7 per cent of world share		Stronger areas: >11 per cent of world share	
	% Share		% Share
Clinical medicine-pharmacy	1.2	Anaesthesiology	18.4
General chemistry	3.7	Pathology	15.6
General physics	4.3	Tropical medicine	15.1
Metallurgy	4.6	Ecology	14.6
Nuclear and particle physics	4.6	Veterinary medicine	13.3
Optics	4.6	General and internal medical research	13.0
Polymer research	5.5	Inorganic and nuclear chemistry	13.0
Oceanography and limnology	5.6	Probability and statistics	13.0
General mathematics	5.7	Gastroenterology	12.9
Ophthalmology	5.7	Dermatology and venereal disease	12.7
Radiology and nuclear medicine	5.7	Electrical engineering and electronics	12.7
Chemical engineering	5.9	Hygiene and public health	12.5
Nuclear technology	6.0	Dentistry	12.2
Solid-state physics	6.0	Microbiology	12.0
Applied physics	6.1	Geology	11.9
Agricultural and food science	6.4	Haematology	11.8
Cardiovascular research	6.7	Botany	11.3
General biomedical research	6.8	Organic chemistry	11.3
Analytical chemistry	6.9		
Surgery	6.9		

Table 6 Major recent trends in British share of world scientific publications, 1977-82

Increase of ≥ 15 per cent in world share, 1977-82		Decline of ≥ 15 per cent in world share	
	% Increase		% Decrease
Entomology	70	Metallurgy	34
Computer technology	61	Clinical medicine-pharmacy	32
Dentistry	17	Materials science	31
Gastroenterology	17	Chemical engineering	26
Geology	16	Tropical medicine	26
Immunology	16	Nuclear and particle physics	26
Haematology	15	Cell biology, cytology and histology	24
		Polymer research	24
		Veterinary medicine	23
		General physics	22
		Electrical engineering and electronics	21
		Applied chemistry	19
		Ecology	19
		Applied physics	17
		Pathology	17
		Botany	16
		Dermatology and venereal disease	16
		Nutrition and dietetics	16
		Endocrinology	15

world citation share have tended to be in fields where Britain has declined most, biomedical research, physics and engineering in particular.

One finding from our previous analysis of earlier NSF/CHI data was that the weakest parts of British basic science are heavily concentrated in areas with strategic technological importance for the future. This finding is reinforced by the updated statistics reported in Table 5. This table lists those scientific specialities in which Britain had a world publication share of more than 11 per cent or less than 7 per cent in 1982. One can see that the areas of strength are mainly in medical and biological specialities, although organic and inorganic chemistry, and electrical engineering and electronics are also quite strong. In contrast, the weaker areas are generally in more applied scientific and engineering specialities such as metallur-

gy, optics, polymer research, chemical engineering and applied physics. What is particularly alarming is the continuing rapid decline in several of these areas; in just two years since 1980, Britain's world publication share in optics dropped from 5.5 per cent to 4.6 per cent, in polymer research from 6.3 to 5.5 per cent and in chemical engineering from 6.7 to 5.9 per cent.

More systematic evidence on recent trends is contained in Table 6, which shows the research areas where Britain has either increased or decreased its world publication share by over 15 per cent in the five years up to 1982. With the exception of computer technology, the overall picture is again one of major declines in areas of science likely to prove strategically important in the future—for example, materials science, applied chemistry and physics, and electrical engineering and

electronics.

We have argued elsewhere that companies in both traditional and new industrial sectors are becoming increasingly dependent on the results of research for maintaining innovative activity and international competitiveness (see *Foresight in Science: Picking the Winners*, Pinter, London, 1984). The figures on recent trends in British science, especially those for more applied scientific and engineering research, thus give major cause for concern, particularly when set in the context of the dramatic fall that has already occurred over the last decade or so in the country's relative technological standing. One indication of the latter is the British share of foreign patents registered in the United States. OTAF data shows that, in the past 20 years, this has declined from around 20 per cent to 8 per cent, while Japan's share has, over the same period, risen from under 10 per cent to over 35 per cent, and West Germany's has remained broadly constant at about 25 per cent, as has that of France at 8 per cent. Clearly, if Britain is to compete more effectively in the international technology market, one important precondition will be an increase in the strength of its underlying research infrastructure.

Optimism

It is therefore worth looking more closely at what has happened since 1980 to determine whether there is any evidence that the long-term decline in British science shows any sign of being reversed. As noted earlier, the figures relating to the British world share of publications do initially seem to provide some grounds for optimism. From the figures in Table 1, one can see that, while the overall share fell from a peak of 9.5 per cent in 1975 to 8.3 per cent in 1980 (a fall of 14.5 per cent in five years or nearly 3 per cent per annum), the British share has remained approximately constant since 1980. Does this herald a renaissance in British science?

There are several possible explanations for this levelling-off in the relative output of British publications since 1980. One is that British scientists, perhaps imbued with a new sense of economic realism engendered by the policies of the Conservative government after its election in 1979, realized the need to increase their productivity and began to publish more research articles in international journals.

In our view, however, a more plausible explanation relates to the funds received by science. Most British academic research is supported by grants from the five research councils with indirect support provided by the University Grants Committee. Figures on research council expenditures in constant prices (calculated using the official EEC research and development price deflator) for 1970 onwards have been plotted graphically in Fig. 1, the expenditure for each year being

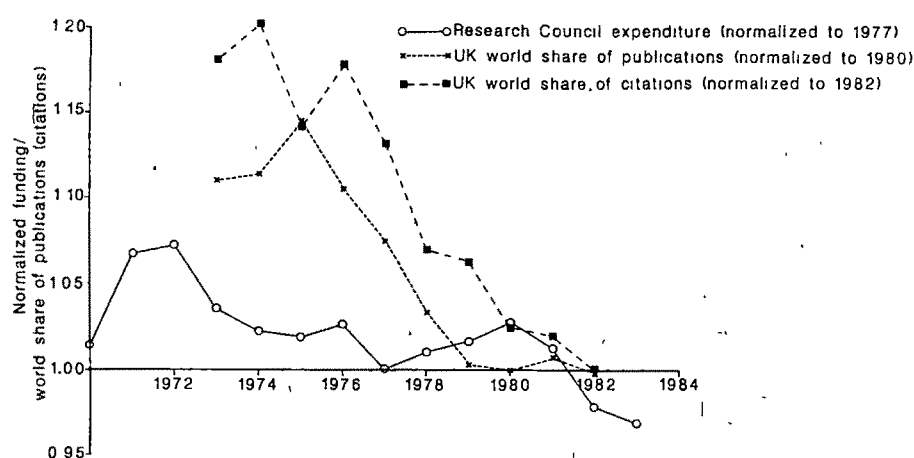


Fig. 1 Association between research council expenditure and UK world share of publications/citations. Source: Tables 1 and 2 on pp.6 and 19 of Butler, E. B. (ed.) *Research and Development in the UK* (Technical Change Centre, London, 1984), which are in turn based on figures published by the Central Statistical Office of the United Kingdom.

expressed as a ratio of that in 1977. As can be seen, research council expenditure grew in real terms between 1970 and 1972, before declining gradually to a minimum in 1977. In the three years up to 1980, expenditure grew slowly but steadily, but since then has fallen sharply in real terms.

Figure 1 also plots the changes in Britain's world share of publications and citations. In the case of publications, there was a peak in 1975 (three years after the peak in funding), but then a rapid fall reaching a minimum in 1980 (again three years after the trough in funding); 1981 registered a modest increase, while in 1982 the British world share began to fall again. There is thus a certain similarity between the trends over time in funding and national share of publications. It would appear that the 6 per cent increase in funding between 1970 and 1972 was reflected in the 3 per cent increase in Britain's share of publications between 1973

and 1975, while the 7 per cent decline in funds in the five years up to 1977 was associated with the sharp drop of 14 per cent in publication share between 1975 and 1980 (see Table 7). In other words, there seems to be a time lag of approximately three years between changes in expenditure and publication share. (This is the reason why the figures on publications have been normalized to 1980 compared with 1977 for funding.) Similarly, there is some suggestion of a time lag be-

tween publication and citation world shares, with the rapid fall in the former between 1975 and 1979 apparently being mirrored in a similarly sharp decline in the latter between 1976 and 1980. If there is indeed an association between research expenditure and the British share of world publications, this would suggest that the levelling off in publication output was largely the result of the 3 per cent increase in research expenditure between 1977 and 1980. Further, if such an association were to continue to hold in the future, one would expect the drop in funding since 1980 to be followed some three years later by a sharp decline in the UK share of publications. It will be interesting to see whether this is actually the case when the CHI/NSF data for 1983-84 become available in 1986. Furthermore, according to the ABRC's latest report, research council funds have continued to fall in real terms since 1983 (by perhaps 1 per cent per annum) and, on the basis of government estimates, will probably continue to do so over the rest of the decade. If the past is any guide, it seems that the UK world share of publications may decline at a somewhat faster rate over the foreseeable future.

Much more research is obviously needed to establish the existence of a direct relationship between funding and publication output and also to explore the reasons behind the fall in the British share of citations. (One reason, for example, may be the widely reported shortage of front-line research equipment in universities and research council laboratories.) Nevertheless, it is clear that one can take little comfort from what has happened since 1980 — the evidence suggests that the levelling-off in the decline of the UK world share of publications between 1980 and 1982 will prove only a very temporary phenomenon.

Over the last year, there has been a considerable growth in concern in Britain with the state of science. At the invitation

of ABRC, the Royal Society is undertaking a study of the health of British science which may provide some evidence on the extent to which the pessimistic conclusions following from bibliometric data of the type presented above are consistent with the views of the scientific community. The Secretary of State for Education and Science is, according to his speech during the 14 June House of Commons debate on science policy, awaiting the completion of that study before making his judgement about whether the quality of science in Britain has declined. Others, however, are already extremely concerned. For example, John Harvey-Jones, chairman of ICI, had this to say about a thorough review by the company of where its next science-based innovations would come from: "I think there is some evidence that the United Kingdom is sliding in the world pecking order. The evidence is still tenuous, but it has led us to start increasing our links with overseas universities that we see as centres of excellence. . . . If our suspicions are correct that the United Kingdom is no longer up at the front as it was, then that presents, in the long haul, very dangerous problems for us." (Quoted in *The Guardian*, 19th February 1985, p.24.) As one of the Members of Parliament who spoke earlier in the parliamentary debate warned, "this may be the last chance. . . to stop the catastrophe that we face as a scientific and educational nation".

We acknowledge support from AGREF, the working group convened by a number of the learned societies, and from the Leverhulme Trust and the Economic and Social Research Council, as well as assistance provided by Dr F. Narin of CHI Research. □

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Table 7 Association between research council expenditure and UK world share of publications

Research council expenditure	UK share of publications
1970-72 +6%	1973-75 +3%
1972-77 -7%	1975-80 -14%
1977-80 +3%	1980-82* 0%
1980-83 -6%	1983-86 ?

* 1983 figure not yet available

and 1975, while the 7 per cent decline in funds in the five years up to 1977 was associated with the sharp drop of 14 per cent in publication share between 1975 and 1980 (see Table 7). In other words, there seems to be a time lag of approximately three years between changes in expenditure and publication share. (This is the reason why the figures on publications have been normalized to 1980 compared with 1977 for funding.) Similarly, there is some suggestion of a time lag be-

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A 150,000-year climatic record from Antarctic ice

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During much of the Quaternary, the Earth's climate has undergone drastic changes most notably successive glacial and interglacial episodes. The past 150 kyr includes such a climatic cycle: the last interglacial, the last glacial and the present holocene interglacial. A new climatic-time series for this period has been obtained using $\delta^{18}\text{O}$ data from an Antarctic ice core.

ALTHOUGH continuous records of climatic and environmental conditions extending beyond 150 kyr are preserved in polar ice sheets, a reliable time series covering the whole of this climatic cycle has not yet been derived from ice cores. This results from logistic reasons, the favourable sites being located in the central regions of Antarctica and Greenland, and the technical difficulty of deep drilling.

The Quaternary has undergone successive glacial and interglacial episodes¹⁻³. Since the first Camp Century drilling in north-west Greenland⁴, five deep-ice cores reaching back to the last glacial have been recovered in Antarctica at Byrd⁵, Vostok⁶ and Dome C⁷ and in Greenland at Dye 3⁸. They have been extensively studied for their oxygen-18 content (denoted $\delta^{18}\text{O}$ expressed with respect to SMOW) and their chemical impurities, which provide valuable information about the Earth's palaeoclimate and palaeoenvironment (see refs 9, 10).

Of these cores, Camp Century seems to extend back to the last interglacial. As it was drilled down to the bedrock, however, this last interglacial ice is confined to the last few metres of the core, because of the thinning of the ice layers during their sinking in the ice sheets. The complexity of the flow lines makes it difficult to date ice cores reaching the bedrock by flow modelling only. Indirect dating techniques, based on the comparison of $\delta^{18}\text{O}$ profiles in ice cores and in deep-sea cores have recently been used⁷⁻⁹ for this purpose. A new Camp Century timescale was proposed which confirms the presence of last interglacial ice and, accordingly, indicates that the deepest part of the Dye 3 ice core was probably deposited during this period⁹. This is possibly also the case for the Devon Island core drilled through a shallow ice cap in the Canadian Arctic¹¹.

We have determined a new climatic-time series obtained from $\delta^{18}\text{O}$ analysis of a 2,083-m ice core drilled at Vostok Station by the Soviet Antarctic Expeditions. The site is located on the high Antarctic Plateau, where the ice thickness is ~3,700 m. The isotope profile covers the last 150 kyr, back to the ice age which preceded the last interglacial, and has essentially been undisturbed by flow conditions.

Vostok ice core

The Soviet Antarctic station of Vostok is located in East Antarctica (78°28' S and 106°48' E) at an altitude of 3,488 m (mean annual pressure 624 mbar; mean annual temperature -55.5°C). The present snow accumulation is between 2.2 and 2.5 g cm⁻² yr⁻¹ (ref. 12) as determined independently from a profile of artificial β activity¹³ and detailed stake measurements¹⁴.

The first series of drilling was performed down to 950 m in successive steps between 1970 and 1974. This 950-m core covers all the Holocene and part of the last glacial periods as shown by the $\delta^{18}\text{O}$ profiles obtained by Barkov and co-workers^{6,15,16}. In 1980, the drilling of a second deep hole was started which

has reached a depth of 2,083 m. The $\delta^{18}\text{O}$ measurements were completed down to 1,412 m (refs 17, 18), a depth which would correspond, from a preliminary dating, to 115 kyr BP (ref. 19).

The collaboration between Soviet and French scientists which centred around the study of this new 2,083-m Vostok ice core was initiated in 1982. Within this framework, sampling was done in the field during the 1982-83 Austral summer and pieces of ice core were sent to Grenoble. The core analysis now in progress in various Soviet and French laboratories concerns, in particular, the chemical analysis of impurities and gas bubbles including CO₂.

$\delta^{18}\text{O}$ profile

Sampling of ice for isotope analysis was done in the field (1982-83 Austral summer) by cutting a continuous slice from the length of the ice after careful cleaning. Sampling was performed on 1.5-2 m ice increments. Samples were sent in solid form to Grenoble and then melted before isotope analysis in Saclay. Two independent series of samples were obtained: (1) a discontinuous series, duplicated to check reproducibility, with one sample taken each 25 m from the surface down to the bottom of the core; (2) a continuous series between 1,406 and 2,083 m.

Oxygen-18 and deuterium determinations were simultaneously performed on all the samples. Here we will discuss the $\delta^{18}\text{O}$ results. The deuterium and deuterium-excess²⁰ results will be discussed elsewhere. The results, obtained with an accuracy of $\pm 0.15\%$ (w.r.t. SMOW), are shown in Fig. 1 as a function of the sample depth. The two sets of samples from the discontinuous series are in good agreement all along the core and their average value at each level is given. For the sake of discussion a continuous line was drawn between each point. There is excellent agreement between the continuous and discontinuous series over all the common parts indicating that the 25-m sampling interval is fine enough to extract reliable palaeoclimatic information. On the other hand, the present data are not detailed enough to document for the Southern Hemisphere such features as the abrupt environmental changes revealed in the Dye 3 ice core between 30 and 40 kyr BP (refs 9, 21).

Down to 1,400 m, the basic features of the $\delta^{18}\text{O}$ curve (Fig. 1) are similar to previously published Vostok $\delta^{18}\text{O}$ profiles^{17,18}, with the last glacial maximum around 400 m, and a $\delta^{18}\text{O}$ shift between glacial and post-glacial conditions of ~5%. However, there is a systematic shift towards lower values for the present set of data of ~3%. Such a shift has already been noticed by Dansgaard *et al.*²².

The $\delta^{18}\text{O}$ profile presented here gives a complete picture of the last climatic cycle from the end of the previous glaciation. In particular, the last interglacial with the highest $\delta^{18}\text{O}$ values around 1,825 m can now be examined in detail. For a further

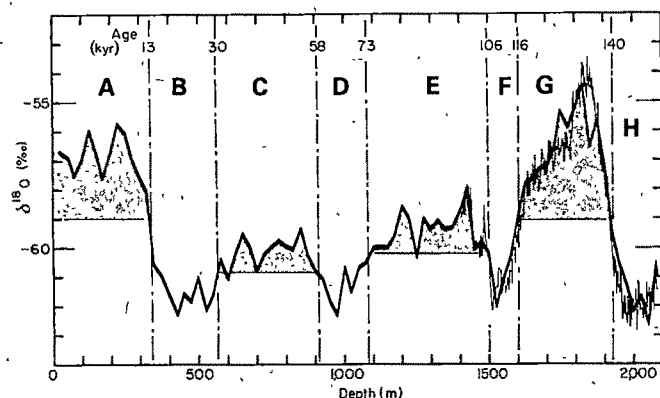


Fig. 1 Oxygen-18 versus depth in the Vostok ice with the definition of the successive stages and indication of the ages corresponding to the limits between these stages. The thick line is drawn from discontinuous data (one for each 25 m), the thin line corresponds to a continuous sampling between 1,400 and 2,083 m. For explanation of A-H, see text.

description of this curve and comparison with other palaeoclimatic profiles, we will designate the successive warm and cold stages consecutively from the top of the record downwards. A striking feature of the profile is the existence of four very well-marked cold periods (that is, low $\delta^{18}\text{O}$) around 425, 975, 1,525 and 2,050 m with practically the same $\delta^{18}\text{O}$ values of around -62% . This makes the definition of the successive stages shown on Fig. 1 relatively straightforward. Starting from the top, the warm periods (hatched areas), are designated by A, C, E and G and the cold periods by B, D, F and H. Stages A and G have the isotopically richest values lying around -57 and -55% , respectively. From the solid curve (Fig. 1), one can note a sharp isotope transition ($\sim 2\%$) inside the G stage at a depth of 1,790 m. Stages E and C are relatively warm interstadial intermediate between full glacial and interglacial conditions.

Dates for the limits between the successive phases are indicated on the top of Fig. 1 (the dating method used is described below). Stage A corresponds to the present Holocene period. Stages B-F cover the last glacial. Stage G includes the peak of the last interglacial and stage H is the last part of the previous glacial.

Ice-core chronology

Many techniques, including isotope and chemical stratigraphies and flow modelling, have been used over the past 10 yr to establish dating of the Vostok ice^{16,19,23,24}. Further detailed isotope study showed that seasonal $\delta^{18}\text{O}$ variations are rapidly smoothed by diffusion²² indicating that reliable dating cannot be obtained from isotope stratigraphy. Unlike the Camp Century core, where the existence of interglacial ice relies on a comparison with deep-sea core isotope profiles, we propose to establish the timescale using an ice-flow model which is independent of other palaeoindicators.

There are two problems with this type of dating. First, for each depth the rate of accumulation at the time and in that area of origin of snow, $A(z)$, must be determined. Second, the thinning function, that is, the ratio of the thickness of a layer to its initial thickness, has to be calculated. We now describe our approach to these problems.

To estimate the past accumulation rate we adopt a procedure based on the observation that the present precipitation rate on the Antarctic plateau is strongly correlated with surface temperature. Robin²⁵ has suggested that this correlation exists because precipitation is governed by the amount of water vapour in the atmosphere circulating above the surface inversion layer. As discussed by Lorius *et al.*⁷ and Jouzel and Merlivat²⁶, there is a link between the temperature of formation of precipitation and its oxygen isotope ratio. Assuming that the same relationship has held in the past, it is thus possible to estimate past accumulation rates from the $\delta^{18}\text{O}$ profile of the ice. Raisbeck *et al.*²⁷ and

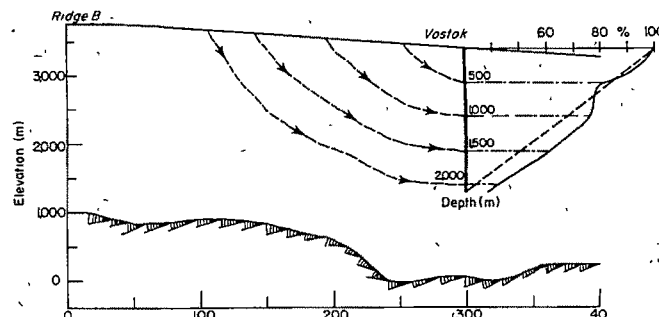


Fig. 2 Surface and bedrock topography along the Ridge B-Vostok axis (lower curve) and the thinning function (top right) expressed as a percentage of the initial thickness, with (solid line) or without (dotted line) taking into account this topography.

Yiou *et al.*²⁸ in this issue have noted that the ^{10}Be concentrations of ice at Dome C and Vostok are consistent with accumulation rates deduced by such procedures. We thus estimate the accumulation function in the following way. First, the temperature of formation of precipitation, T_F , is estimated along the profile as described by Lorius *et al.*¹⁰. Then, the accumulation rate, $A(z)$, is calculated from the product of its present value, $A(0)$, times the ratio of the derivatives of the saturation vapour pressure—with respect to $T_F(z)$ for the time of precipitation and with respect to $T_F(0)$ for the present conditions. This formulation is derived from a simplified unidimensional model neglecting the possible changes in circulation intensity above the area of precipitation.

The use of a two-dimensional glaciological model is well suited to the Vostok area as the ice flowlines are roughly parallel in this region of East Antarctica²⁹. The change of the ice thickness upstream of the drilling site is important because of the bedrock topography³⁰ (Fig. 2) and this has been taken into account when using the analytical model of Lliboutry³¹. Finally, ice-core dating is established using an iterative process. Starting from a plausible dating obtained from a simple one-dimensional model the ice origin is determined. Then, the thinning function is calculated and the ice redated all along the core. The process is repeated using the new dates as the initial conditions until the initial and final datings converge.

To illustrate the influence of the bedrock topography, the thinning functions with (Fig. 2, solid line) and without (Fig. 2, dotted line) taking into account this topography can be compared. This demonstrates the strong effect of bedrock topography between 500 and 1,000 m. For these calculations, ice velocities were approximated by their average values using mass continuity over the entire climatic cycle.

The results are presented in Fig. 3 for three values of the present accumulation. The annual accumulation (expressed with respect to its present value) is obviously correlated to the $\delta^{18}\text{O}$ profile with lower values during glacial periods as previously stated from Dome C ice-core studies^{7,27} and independently proposed at Vostok from ^{10}Be measurements²⁸.

From available data¹², an average value of $2.3 \text{ g cm}^{-2} \text{ yr}^{-1}$ was adopted for the present accumulation rate, giving ages of 98 kyr BP at 1,412 m (that is, significantly younger than the previously estimated¹⁹ 115 kyr BP) and of ~ 160 kyr BP for the bottom of the core.

This accumulation value, although uncertain given the very short period (<10 yr) over which this parameter was determined, seems to be a reasonable average over the Holocene period. Our dating puts the end of the last climatic transition at around 10 kyr BP which compares well with the same event recorded in the Dome C East Antarctic core at ~ 10.5 kyr BP (ref. 7). The uncertainty on this value is thus $\sim 5\%$, a value which would give a lower limit for the relative dating uncertainty all along the core. Dating of the first part of the core is also indirectly supported by the existence of a dust content maximum between ~ 13 and 26 kyr BP (ref. 32), a period of aridity over Australia³³.

However, other sources of errors come from estimations of accumulation changes and, to a lesser degree, of the thinning function. Thus, the age of the bottom of the core is probably not known with a precision better than 10–15 kyr.

Palaeoclimatic information

The isotope content of polar snow is primarily governed by T_F , but it also depends^{9,10,34,35} on several other parameters such as the origin and dynamical history of the air masses and the microphysical processes leading to snow formation. A way of reconstructing the full, space-time variations of isotope contents of vapour and precipitation is to incorporate the isotope cycles in a general circulation model of the atmosphere (GCM)³⁶.

Until this new approach is fully developed, the climatic interpretation must be based on $\delta^{18}\text{O}$ -temperature relationships observed in modern precipitation and/or isotope models which give only a simple schematic overview of the dynamic history of the air masses. Although aware of the limitations, we are relatively confident that when the two methods are combined major temperature changes, such as those accompanying glacial-interglacial transitions, can be estimated. This is especially true for central East Antarctica for several reasons. First, the observed $\delta^{18}\text{O}$ -temperature relationship³⁷ is quite linear (correlation coefficient, $r=0.989$ for the -20 to -55°C interval) and its slope can be correctly explained on a theoretical basis applying a recently developed kinetic isotopic model²⁶. Second, the ice thickness has probably undergone only minor fluctuations and the ice origin can be reliably reconstructed. In addition, our confidence in a temperature reconstruction is reinforced in the Vostok core by the good agreement between accumulation changes derived by transforming the $\delta^{18}\text{O}$ profile into a temperature profile and those derived from ^{10}Be (ref. 28).

After correcting for the change in $\delta^{18}\text{O}$ of the oceanic water (maximum 1.6%)³, this temperature reconstruction is carried out as in ref. 10. With this interpretation, the glacial-interglacial surface temperature shifts are $\sim 10^\circ\text{C}$ for the H-G transition ($\delta^{18}\text{O}$ shift of 6.5%) and 8°C for the B-A transition ($\delta^{18}\text{O}$ shift of 5%). C and E interstadials are around 2 and 4°C , respectively, warmer than full glacial conditions and the four cold stadials H, F, D and B are within $\sim 1^\circ\text{C}$. The peak of the last interglacial maximum is significantly warmer than the Holocene period. The difference is estimated to $\sim 3^\circ\text{C}$, 1°C of this being due to the formation of the interglacial ice 100 km further inland (Fig. 2). All these values do not take into account the possible variations in the ice-cap altitude. These corrections will be done, if necessary, from the total gas content which is under investigation. Preliminary data (D. Raynaud, personal communication) suggest that the isotope signal is essentially of climatic origin which is independently corroborated by some studies tending to demonstrate the relative stability of the East Antarctic ice sheet at the considered timescale^{38,39}.

Comparison with other ice cores

Concerning the transition between the last glacial maximum (around 18 kyr BP) and the Holocene periods (B-A in our terminology), the present results confirm the excellent agreement between the two East Antarctic sites, with a $\delta^{18}\text{O}$ shift, before corrections, of 5.4% at Dome C⁷ and of $\sim 5\%$ at Vostok, and to a lesser degree with the Byrd West Antarctic site (shift of 7%)⁵. The inferred surface temperature change for Antarctica is ~ 8 – 10°C . These shifts agree well with those deduced for high southern latitudes from GCM ice-age modelling^{40,41}, although recent simulations predict lower temperature shifts^{42,43}. The isotope shift is also 7% in southern Greenland (Dye 3)^{8,9}. The higher values ($>10\%$) registered in northern Greenland and adjacent regions (Camp Century, Devon Island) can be partly attributed either to a change in the elevation of the ice formation⁴⁴ or to a more drastic climatic change in this area than in southern Greenland or in Antarctica⁹.

For the last glacial, a detailed comparison with other ice cores is difficult because only 7% of the Vostok core was analysed down to 1,400 m. In any case, it would be necessary to discuss

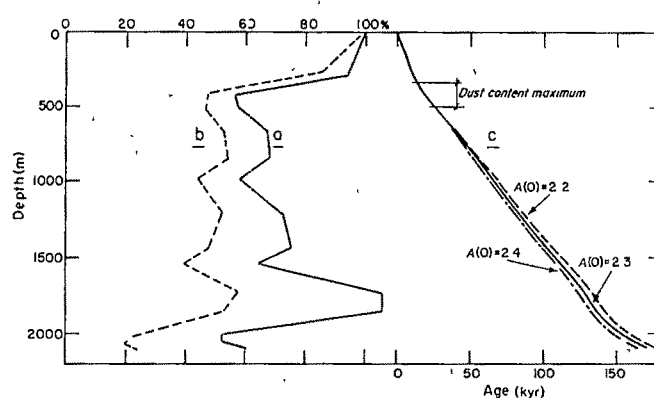


Fig. 3 a, Initial thickness of annual layers (accumulation rate $A(z)$); b, thickness of these annual layers in the core. Both a and b are expressed with respect to the present (accumulation rate $A(0)$); c, depth-age relationship for different values of $A(0)$ in $\text{g cm}^{-2} \text{yr}^{-1}$.

thoroughly the successive timescales derived for the Byrd, Camp Century and Dye 3 cores. Such a comparison will be undertaken in the near future when a continuous profile becomes available for the Vostok core.

The apparent time elapsed between the interglacial peak and the following $\delta^{18}\text{O}$ minimum is more than twice as long at Vostok than at Camp Century. This is not strictly an ice core feature and cannot be discussed further here (see below) as the Camp Century timescale is deduced from a comparison with the deep-sea record⁹. This shows the difficulty of applying such an indirect dating method.

Comparison with $\delta^{18}\text{O}$ in deep-sea cores

It is now widely accepted that $\delta^{18}\text{O}$ in benthic foraminifera primarily reflects ice-volume changes^{3,45,46}. Although the relationship can be distorted by many factors, the CLIMAP project members concluded that there is no compelling evidence to reject the first-order assumptions of isotope synchronicity or fidelity to the ice volume⁴⁵. Although varying in detail, all the cores give similar general trends^{3,47}, allowing a common definition of isotope stratigraphy. The period of interest here covers Emiliani's stages 1–6 (ref. 48), stage 5 being subdivided in 5a to 5e (ref. 49), as we have illustrated on Fig. 4e.

With the adopted Vostok dating, there is a good correspondence between stages 1–4 and Vostok stages A–D. Stage E covers 5a–5c and F corresponds to 5d. However, there is no clear correspondence between 5e and G representing the last interglacial in deep-sea and ice records, respectively (Fig. 4). There is a major difference in the duration of these two stages; 11 kyr for 5e and 24 kyr for G. Also, the last interglacial peak in the Vostok core is around 130 kyr BP whereas the corresponding ice-volume minimum in the deep-sea record is dated earlier at 122-kyr BP (ref. 45).

A doubling of the accumulation rate over this period would lead to a good correspondence between the 5e and G stages. While this possibility cannot be completely ruled out, there is no indication of such a drastic change in the precipitation regime. Part of the time-lag between the two peaks could be real (~ 3 kyr) as already observed between Southern Hemisphere ocean temperature and ice volume records but dating uncertainties (probably of ~ 10 kyr at this level) prevent a detailed discussion of this possible lag. On the other hand, the fact that ice stage G probably lasted longer than stage 5e is a key element for understanding the last interglacial climate and the initiation of the last ice age. A detailed interpretation is beyond the scope of the present study and would require knowledge of the exact timing between the temperature and ice volume signals. Recent measurements of $\delta^{18}\text{O}$ of air entrapped in ice cores could provide a way of correlating deep-sea and ice-cores $\delta^{18}\text{O}$ profiles and then establishing this timing⁵⁰.

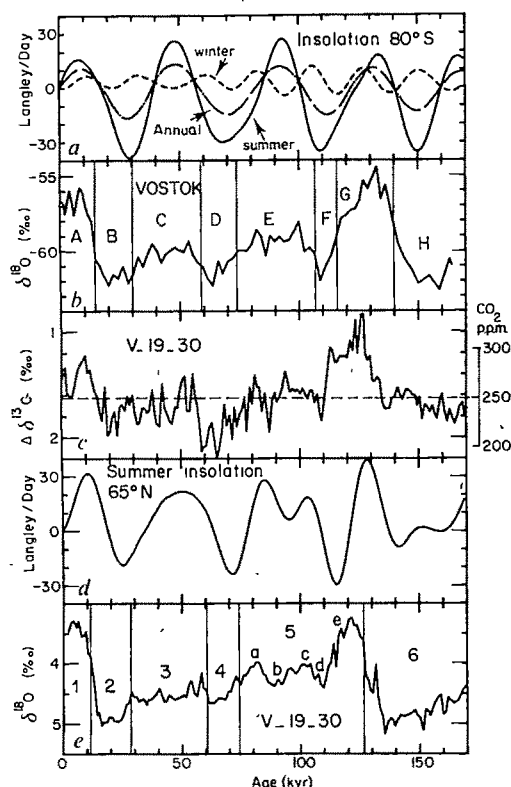


Fig. 4 Variation with time of: a, winter, summer and annual insolation at 80° S (ref. 59); b, $\delta^{18}\text{O}$ in the Vostok ice (this work); c, $\Delta\delta^{13}\text{C}$ in the V 19-30 deep-sea core⁶⁸ and indication of the CO_2 scale on the right; d, summer insolation at 65° N (ref. 59); e, $\delta^{18}\text{O}$ in the V 19-30 deep-sea core⁶⁸ for *Uvigerina Senticosa* with an indication of the successive climatic stages.

According to CLIMAP members⁴⁵, the last interglacial was similar to the current climate. However, they noted some indications of a slightly warmer climate. The Vostok record brings quantifiable evidence of atmospheric warming over Antarctica during this period. This is useful information because there is some controversy about the behaviour of the East Antarctic ice sheet during the last interglacial⁴⁵. In addition, the Vostok record does not support an interglacial character for marine stage 5c (first part of E), an argument used to suggest an East Antarctic ice surge around 95 kyr BP (ref. 51).

Whereas glacial buildups in the deep-sea record (Fig. 4e) are gradual giving the ice-volume cycles their asymmetrical saw-toothed character⁵², the ice record (Fig. 4b) shows that the minimum temperatures were of the same order during cold periods (H, F, D and B stages). This may be linked to the rapid response of the atmospheric system to forcings and feedbacks and also suggests that there is a very stable mode of the climatic system for these cold periods. Such differences in shape were previously noted between ice-volume and temperature signals recorded in some oceanic cores⁵³.

Forcing and feedback factors

Beyond this reconstruction of the East Antarctic climate, we now discuss some current important aspects of the mechanisms which have probably controlled this climate and how the East Antarctic climate is related to the global climate. We will examine the possible role of changes in insolation, atmospheric CO_2 content and global ice volume.

Insolation changes. Over recent years, the Milankovitch hypothesis that orbital variations are the principal causes of the Pleistocene ice ages has received considerable support^{45,52-54}, and has stimulated many modelling efforts⁵⁵⁻⁵⁸. The spectral analysis of proxy records has revealed periodicities statistically correlated to the precessional (19 and 23 kyr), obliquity (41 kyr) and eccentricity (100 kyr) cycles. There is a general consensus

that summer insolation at about 65° N might be a plausible forcing as Northern Hemisphere ice sheets have undergone the major changes, and that the dominant 100-kyr cycle would result from a nonlinear response of ice sheets to orbital forcing.

Before discussing the possible link between the Northern Hemisphere insolation changes and the Vostok climate, we will investigate whether there is a relationship between this climate and the local changes of insolation.

The comparison of the $\delta^{18}\text{O}$ ice profile (Fig. 4b) with the annual insolation curve for 80° S (ref. 59) (Fig. 4a) reveals a very striking relationship between the cold and warm isotope stages and the minima and maxima of the insolation curve. While discontinuous sampling prevents us from performing reliable spectral analysis, especially for short periodicities, this remarkable correlation strongly supports the role of orbital forcing in climatic change.

The particular importance of the obliquity cycle, previously suggested from some high-latitude oceanic cores⁵⁴, would indicate that orbital forcing acts at a local scale. At 80° S, the maximum change of annual insolation is ~7% (ref. 59) which, from the radiative equilibrium equation, corresponds to a direct temperature effect of 4 °C. This direct effect is certainly important when compared with the 10 °C temperature range inferred from the $\delta^{18}\text{O}$ profile.

The apparent climatic synchronicity between the Northern and Southern Hemispheres poses a problem because the seasonality history of the polar insolation at 65° is 'different' for the two hemispheres^{60,61}. One explanation⁶¹ could be that, for polar regions, the climate responds to annual insolation changes that are in phase for the two hemispheres. The correlation between the 80° S annual insolation and the $\delta^{18}\text{O}$ record gives some support to this hypothesis.

Finally, accepting the role of orbital forcing on the Southern Hemisphere climate, the existence of a roughly 40-kyr cycle in the $\delta^{18}\text{O}$ ice core record indirectly supports the Vostok core dating.

Atmospheric CO_2 concentration. CO_2 measurements from the analysis of air bubbles entrapped in ice from Greenland and Antarctic cores have shown that CO_2 changes are associated with climatic changes. Such direct measurements are available for the past 40 kyr (refs 62-65) and show a CO_2 increase of ~40% between the last glacial maximum and the pre-industrial Holocene period. These measurements suggest that CO_2 and its greenhouse warming might be involved in the control of the ice ages²¹ and also in their interhemispheric synchronicity^{42,60}.

Shackleton *et al.*⁶⁶ have used the idea that the difference in carbon isotope ratios in surface and deep oceanic waters varies with CO_2 levels⁶⁷ to develop an indirect approach which extends the atmospheric CO_2 record further back in time. They estimate this parameter from the $\delta^{13}\text{C}$ difference between planktonic and benthic foraminifera ($\Delta\delta^{13}\text{C}$). The validity of this approach is supported by the fact that the estimated CO_2 values are consistent with the polar record over their common part⁶⁶ (the past 40 kyr). However, because many assumptions underlie this method, it has to be corroborated either from other oceanic cores or, more directly, from ice cores. A study is in progress to obtain a detailed CO_2 profile along the Vostok core to examine directly the CO_2 -climate connection over the past 150 kyr. In the following discussion, we will treat the $\Delta\delta^{13}\text{C}$ curve as a CO_2 curve.

The first 150 kyr of this $\Delta\delta^{13}\text{C}$ record⁶⁸ is shown on Fig. 4c. One can easily see the similarities between this curve and the $\delta^{18}\text{O}$ Vostok record. A relationship between CO_2 content and atmospheric temperatures is expected. Indeed, climate models predict that the greenhouse CO_2 effect should be amplified in high latitudes especially near ice margins^{69,70}. Thus, these observed similarities suggest a CO_2 -atmospheric temperature connection all over the last climatic cycle. According to Stauffer *et al.*²¹ a 40% CO_2 change corresponds to a temperature rise of 2-4.5 °C in high latitudes. Thus, the hypothesis that CO_2 changes are an important factor in controlling high latitude climate is well within the realm of possibility.

There are several other features of the CO₂-climate relationship. CO₂ measurements at Dome C indicate that the increase in CO₂ concentration associated with the end of the last ice age began before, or simultaneously with, the climatic change at high southern latitudes⁶⁵. On the other hand, from the 340-kyr reconstructed CO₂ curve, Pisias and Shackleton⁷¹ inferred that CO₂ lags the orbital forcing (summer insolation at 65° N) but leads the ice volume, by an average of 2.5 kyr. From this, they made two important points^{68,71}: (1) CO₂ changes are not a consequence of climatic changes but may represent part of the mechanisms by which orbital variations induce changes in climate; (2) the presence of a strong obliquity component suggests a high-latitude control for the mechanisms by which CO₂ responds to orbital forcing.

This also puts constraints on the way in which CO₂ levels in the ocean surface, which eventually control the atmospheric CO₂ levels, can vary⁶⁸. Recently, four groups⁷²⁻⁷⁵ have independently explored CO₂ scenarios implying a high latitude control through: the changes in ocean circulation; the influence of the amount of light reaching the polar regions on the utilization of nutrients; and a combination of these two factors.

The Knox and McElroy nutrient scenario⁷⁴ includes a mechanism whereby small changes in insolation at high latitudes could result in large changes in CO₂ and thus looks attractive in view of the possible CO₂ climatic role at the timescale considered here although this type of scenario⁷⁸ is not supported by recent results on planktonic foraminifera from Antarctic deep-sea sediments.

Ice-volume feedbacks. Although many assumptions are involved, the above discussion suggests that local insolation and possibly the atmospheric CO₂ have played a part in determining the Antarctic climate over the past 150 kyr. The two factors alone, however, would not explain all the climatic variability seen in the $\delta^{18}\text{O}$ Vostok record. In particular, we observe that the very cold stage F corresponds to an intermediate inferred CO₂ value.

Note that this stage F corresponds to the time of lowest insolation at 65° N (Fig. 4d)⁵⁹. (Indeed, there are similarities between this insolation curve and the $\delta^{18}\text{O}$ Vostok record (Fig. 4b) over all the past 150 kyr.) Modelling of the growth and retreat of the ice sheets has shown that ice volume changes can be driven by summer radiation in Northern Hemisphere high latitudes^{56,57,76,77}. Although all of the models do not specifically refer to 65° N, the generated ice-volume curves resemble, with a few kiloyears lag, the summer insolation at this latitudinal band. As shown in Fig. 4d, high volumes correspond to low insulations and vice versa. Such generated ice volume curves differ from ice volumes deduced from benthic foraminifera (Fig. 4e). In particular, note the difference during stage F. According to Budd⁶¹ the two approaches of deriving ice volumes can be reconciled if the contribution of ^{18}O -poor Antarctic ice is taken into account. This raises the question of how Northern Hemisphere ice extent can influence Antarctic climate.

Although we cannot answer this question here, we note that recent GCM results⁴² show that such a relationship cannot be explained by the interhemispheric exchange of heat in the atmosphere. Other mechanisms would have to be involved, such as a change in the cross-equatorial heat transport by the ocean circulation^{42,60,78} or a response of the East Antarctic ice sheets to sea-level changes⁶¹. This second hypothesis is valid only if its apparent contradiction with the time lead of Antarctic sea-ice compared with the Northern Hemisphere ice volume can be solved as suggested by Budd⁶¹.

Conclusions

We have obtained the first complete and unambiguous isotope description from a deep-ice core of the last climatic cycle. The 2,083-m Vostok record covers the past 150 kyr extending into the end of the previous glacial period. The timescale has been established by combining a glaciological model appropriate here as the core represents only about the first half of the ice sheet, with an original approach for estimating past accumulation rates from the isotope record. While this method has its own limita-

tions, it has the advantage of being independent of other palaeo-indicators or climatic forcing series.

This $\delta^{18}\text{O}$ record has been interpreted as essentially representing temperature changes over East Antarctica. This interpretation is based on the $\delta^{18}\text{O}$ -temperature gradient observed for modern precipitation and on a dynamically-simple isotope model. Although aware of the necessity of a more detailed modelling of the atmospheric $\delta^{18}\text{O}$ cycle using GCMs, we have confidence in the temperature interpretation as far as major $\delta^{18}\text{O}$ changes are concerned.

In this interpretation, the Vostok record provides extremely rich climatic information. The present Holocene period was preceded by a long glacial period marked by two relatively warm interstadials. The well-marked last interglacial was significantly warmer than the Holocene and the end of the previous glacial was quite similar to the last glacial maximum.

A comparison with $\delta^{18}\text{O}$ in deep-sea cores shows that the Antarctic climate and the global ice volume have not evolved in the same way. The last interglacial appears to be about twice as long in the Antarctic temperature record as in the ice-volume record. In addition, there are marked differences in the shapes of the records during the last glacial. Thus, we now have access to another different, but complementary, climatic record fully covering the last climatic cycle.

The presence of a roughly 40-kyr cycle in the Vostok record strongly supports the role of orbital forcing in determining climatic changes. This record is approximately in phase with the annual insolation at 80° S suggesting that this forcing operates at a local scale. There are also some common features between the climatic curve and the summer insolation at 65° N which may be the result of the influence of the Northern Hemisphere ice sheets on the Antarctic climate.

There are striking similarities between the $\Delta\delta^{13}\text{C}$ curve of Shackleton and co-workers^{66,68} and the $\delta^{18}\text{O}$ record. Following these authors in treating the $\Delta\delta^{13}\text{C}$ curve as a CO₂ curve, there appears to be, over a complete climatic cycle, a relationship between atmospheric CO₂ levels and atmospheric temperatures as previously suggested for the past 40 kyr.

There are several other ways to obtain more climatic information from the Vostok ice than the $\delta^{18}\text{O}$ method described here. Interesting results concerning ^{10}Be have been obtained²⁷, and other studies are either in progress or planned for the near future. They include additional deuterium and oxygen-18 determinations and measurements of total gas volume, crystal size, CO₂ in the air bubbles, $^{18}\text{O}/^{16}\text{O}$ ratio of oxygen in air and aerosol content.

The Vostok ice core offers the possibility of measuring many other parameters and their variation with time. As shown from other ice cores, the number of these parameters of interest is growing. Recent studies include more and more chemical elements⁷⁹⁻⁸³ and trace gases⁸⁴ as well as $^{12}\text{C}/^{13}\text{C}$ ratio in CO₂ (ref. 85). Although their first objective is to reconstruct the Earth's palaeo-environment, some of these parameters also contain, at least indirectly, palaeoclimatic information. Finally, it is hoped to reach greater depths in the Vostok drilling and thus to extend the time scale considerably.

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A phage repressor-operator complex at 7 Å resolution

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The crystal structure of a complex between the DNA-binding domain of phage 434 repressor and a synthetic 434 operator shows that the protein, very similar in conformation to λ repressor, binds to B-form DNA with the second α-helix of a helix-turn-helix motif lying in the major groove.

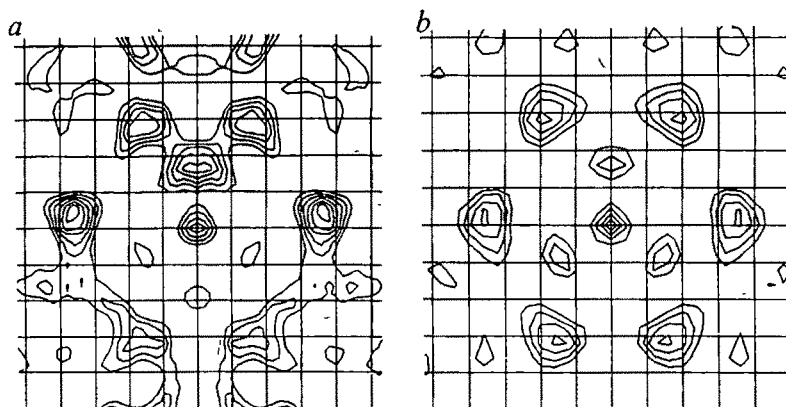
WE present the X-ray crystal structure at 7 Å resolution of a repressor protein bound to its operator DNA. The protein (also a positive regulator of gene expression) is the amino-terminal, DNA-binding domain of the repressor encoded by coliphage 434, a close relative of phage λ. The operator is a 14-base-pair (bp) synthetic oligonucleotide with 2-fold symmetry that is specifically recognized by 434 repressor. Determination of the structure was facilitated by the preparation of isomorphous repressor-DNA crystals using operator DNA with 5-bromodeoxyuridine instead of thymine at two symmetry-related positions. Important aspects of the structure include: (1) The repressor conformation, predominantly α-helical, is remarkably similar to that of the first four α-helices of λ repressor¹. (2) The DNA conformation is B-like, with some deviation at the ends of the operator. (3) Repressor contacts the DNA backbone at several distinct points. Bushman *et al.*² elsewhere in this issue present evidence that these are sites of strong interaction between repressor and DNA phosphates. (4) An α-helix (α3) lies in the

major groove of the DNA and another α-helix (α2) lies across the groove. The helices α2 and α3 correspond to the helix-turn-helix motif found in the crystal structures of three other DNA-binding proteins^{1,3,4} (and postulated to occur in many more, including 434 repressor⁵⁻⁷). The mode of binding to DNA is similar to that proposed for those proteins on the basis of model building^{1,8-10,22}, with the α3 helix disposed so that its residues can make sequence-specific contacts with DNA. Changing these residues in the α3 helix of 434 repressor can change the specificity of the repressor (ref. 11 and accompanying paper¹²).

Structure determination

Crystals of the complex of the 434 repressor DNA-binding domain R1-69 and synthetic 434 operator DNA were prepared as described previously¹³. The space group is I422, with $a = b = 166.4$ Å, $c = 139.4$ Å. Each strand of the synthetic DNA operator had the sequence 5'HO-d(A-C-A-A-T-A-T-A-T-A-T-T-G-T)-OH 3'. Derivative crystals were also prepared, using a similar

Fig. 1 Noncrystallographic Harker section. *a*, The section of the difference Patterson that lies 45.6 Å from the origin and perpendicular to the body diagonal of the unit cell. The body diagonal intersects the section at its centre. The small squares of the grid are 5 Å on a side. The set of six peaks corresponding to vectors between equivalent Br atoms on adjacent 14mers forms a roughly hexagonal array, with each peak lying ~18 Å from the body diagonal. Peaks from analogous Br atoms on straight B-DNA with its helix axis coincident with the body diagonal would lie 9.5 Å from the centre. *b*, The same section after 3-fold averaging about the body diagonal, using the programs of Bricogne¹⁸.



oligonucleotide with 5-bromodeoxyuridine instead of dT at position 7. (Both 14mers were purchased from Pharmacia P-L Biochemicals.) Data were collected to 7 Å resolution using a Xenonics imaging proportional counter¹⁴ with CuK α radiation from an Elliott GX-6 rotating anode X-ray generator. Integrated intensities were obtained as described by Durbin *et al.*¹⁴ and processed using programs from P. R. Evans (MRC Laboratory of Molecular Biology, Cambridge, UK). Combination statistics are shown in Table 1. The mean isomorphous difference was

Table 1 Data combination statistics

	R_{sym}	N	$\bar{I}$
Native	0.087	1,171	270
Br	0.066	1,134	455

N , number of measurements; $\bar{I}$, mean intensity; $R_{\text{sym}} = \sum_h |I_h - \bar{I}_h| / \sum_h I_h$.

small (0.12 for 1,105 common reflections). The consequent noisy difference Patterson could not be interpreted directly, but the positions of the Br atoms were obtained by a procedure that took advantage of the previously described analysis of packing in these crystals¹³.

Protein-DNA complexes in the I422 crystals are stacked in rods with noncrystallographic 3₁ symmetry, parallel to the body diagonals of the unit cell. The rise per repeat of the 3₁ axis is 45.6 Å, so that the section of the difference Patterson perpendicular to the body diagonal and intersecting it 45.6 Å from the origin is a 'noncrystallographic Harker section', in the sense that it contains peaks representing vectors between atoms related by the noncrystallographic symmetry. On this section of the Patterson, we therefore expected to find a set of 3-fold-related peaks corresponding to vectors between Br atoms on different 14mers. Moreover, the known chemical structure of the derivative restricted the range of possible distances of these peaks from the noncrystallographic symmetry axis. Figure 1 shows the pattern actually found, before and after 3-fold averaging. It is similar to the pattern expected on the basis of the packing model of Anderson *et al.*¹³, in which the DNA is stacked end-to-end in straight rods parallel to the unit cell body diagonals, but the peaks are ~5 Å farther from the axis than predicted. We found that B-DNA with a slight right-handed supertwist placed Br atoms in positions that matched the observed pattern much better. The local tilt of the DNA in this model was 12°.

Assignment of the Br-Br vectors on the noncrystallographic Harker section gives coordinates of the Br positions relative to the 3₁ axis, but leaves undetermined one translational degree of freedom, the displacement of this noncrystallographic symmetry axis from the body diagonal of the unit cell. There is also one origin ambiguity (see Fig. 2). The ambiguity was resolved,

and the translational displacement determined, using a search procedure in which calculated Br contributions and centric $[F_{\text{der}} - F_{\text{nat}}]$ were compared (by means of a correlation function) between 15 and 7 Å resolution. The details will be reported elsewhere (J.E.A., J. Moulai and S.C.H., in preparation). A peak in the correlation function gave a trial solution, which was refined by conventional methods¹⁵ (Table 2).

Native phases were determined and used to compute an initial electron-density map, which was inspected on an Evans and Sutherland PS300 interactive graphics system using the program FRODO (ref. 16). The map was noisy and contained no easily recognizable features, but averaging with respect to the noncrystallographic 3₁ axis gave some indication of a double-helical DNA backbone and several rod-like features where protein was expected.

Phases were refined by exploiting the redundancy of information present in the noncrystallographic 3₁ symmetry^{17,18}. In drawing the envelope required for phase refinement^{17,18}, it proved helpful to use a model map that included the N-terminal domain of λ repressor¹ and DNA as a guide. The model consisted of the coordinates of λ -repressor residues 17-85 (ref. 1) (to match the size of R1-69), positioned to fit a straight double helix of DNA aligned along the noncrystallographic 3₁, and, instead of straight DNA, the coordinates of the supertwisted

Table 2 Br atom position refinement

Resolution (Å)	N	m	$f_{\text{rms}}/E_{\text{rms}}$	R_K
13.12-11.67	143	0.46	1.74	0.045
11.67-10.50	189	0.42	1.58	0.061
10.50-9.55	260	0.45	1.75	0.059
9.55-8.75	306	0.42	1.56	0.058
8.75-8.08	362	0.43	1.58	0.078
8.08-7.50	377	0.39	1.53	0.082
7.50-7.00	407	0.38	1.40	0.132
13.12-7.00	2,028	0.42	1.55	0.075

Comparison of initial and refined Br atom positions

Site	Initial	Refined	Occupancy
1	(0.16, -0.20, -0.01)	(0.161, -0.195, -0.013)	0.903
2	(-0.03, -0.26, 0.19)	(-0.033, -0.259, 0.190)	1.001
3	(-0.02, -0.29, 0.23)	(-0.020, -0.289, 0.227)	0.788
4	(-0.21, -0.48, 0.27)	(-0.211, -0.480, 0.270)	0.781
5	(-0.24, -0.47, 0.31)	(-0.242, -0.467, 0.307)	0.960
6	(-0.31, -0.66, 0.51)	(-0.306, -0.661, 0.511)	0.905

Statistics were calculated after 10 cycles of phasing and refinement¹⁵, using I422 data expanded to space group I222, starting with the positions calculated from model B-DNA with a right-handed supertwist ($\alpha = -12^\circ$; ref. 21) of the C-7 atoms of the thymine in position 7 of the 14mer sequence. m , Figure of merit; f_{rms} , root-mean-square heavy atom structure factor; E_{rms} , root mean square lack of closure error; $R_K = \sum |F_{\text{PH}}^{\text{obs}} - F_{\text{PH}}^{\text{calc}}| / \sum |F_{\text{PH}}^{\text{obs}}|$.

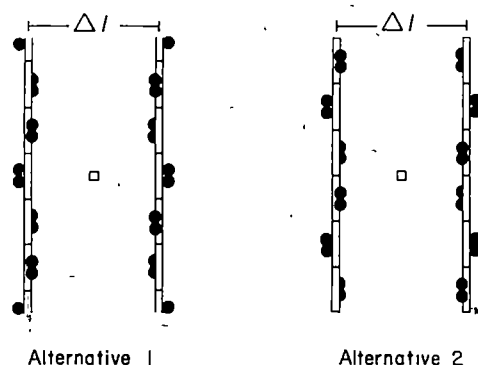


Fig. 2 Schematic projections down the 4-fold axis c (squares) of two of the four rods most closely associated with a lattice point, showing the two possible choices of origin (alternatives 1 and 2). The translational displacement, Δl (one of the parameters searched as described in the text) is shown for each. In alternative 1, the dyad between two Br atoms (filled circles) is coincident with the diagonal crystallographic dyad [110] (dotted line). In alternative 2, the dyad between two operators (rectangles) is coincident with the crystallographic dyad.

DNA used to locate the Br atom positions, with its superhelix axis coincident with the noncrystallographic symmetry axis. Inspection of the electron density computed from 7 Å structure factors of this model helped us to recognize features of the averaged initial map around which to draw the envelope. A total of six cycles of phase refinement were performed. The statistics are shown in Table 3 and part of the final map in Fig. 3. The positions of Br atoms are shown as stippled spheres in Fig. 3. Ribbons of density corresponding to double-helical DNA backbone are clearly visible, and it is easy to distinguish major and minor grooves. Rod-like density features (clearly low-resolution α -helices) are also visible next to the DNA on the opposite side from the Br atoms. One of these lies in the major groove of the DNA, and another is roughly at right angles to it. Their size and relative orientation show that they correspond to the helix-turn-helix motif seen in λ repressor¹, λ cro² and catabolite gene activator protein⁴, and their position on the operator is consistent with modes of binding suggested for those proteins by model building^{1,8-10,22}.

Structure of R1-69

A backbone model for λ repressor¹ can be superimposed on the electron-density map of the co-crystal in such a way that $\alpha 2$ and $\alpha 3$ (the helix-turn-helix motif) are well aligned in rod-like density features (Fig. 4). Inspection shows that two other 'sausages' of density correspond closely to the $\alpha 1$ and $\alpha 4$ helices of λ repressor. The axis of the $\alpha 1$ density is displaced from the axis of the λ -repressor $\alpha 1$ helix by 2–3 Å, and its orientation is slightly different. The N-terminus of 434 repressor $\alpha 1$ density is close to the C-terminus of $\alpha 3$ density, and com-

parison with the λ repressor model suggests that it corresponds to the N-terminus of the polypeptide chain. The fit of the λ -repressor $\alpha 4$ helix to the co-crystal density is even closer than that of $\alpha 1$, although not quite as good as $\alpha 2$ and $\alpha 3$. The final helix of the λ -repressor domain ($\alpha 5$, not shown in Fig. 4) does not lie in clearly rod-like density, suggesting that the fold of the 434 repressor polypeptide chain differs significantly from the λ -repressor fold in this region. The λ -repressor helix $\alpha 5$ mediates a close dimer contact between N-terminal domains. Such a close contact may indeed not occur in 434 repressor, because in the absence of DNA, R1-69 crystallizes as a monomer. (The space group of the R1-69 crystals is $P2_12_12_1$, with one protein molecule per asymmetric unit; S. Almo, J.E.A. and S.C.H., unpublished data.) The links between helices are not very clear in the co-crystal map (only the $\alpha 2$ and $\alpha 3$ 'sausages' are bridged by density at the level we chose to contour), but the correspondence between the λ -repressor model and rod-like density features makes us confident that we have correctly determined connectivity and chain direction. We conclude that R1-69 has a conformation very similar to that of the DNA-binding domain of λ repressor. It contains at least four α -helices, and their orientation and packing are similar in the two structures. The 434 repressor lacks the N-terminal arm of λ repressor, and the C-terminal portion of the domain may follow a different course.

Operator structure

Only the DNA backbone is clearly delineated at this resolution; density corresponding to base pairs is less regular. Ribbons of density follow fairly closely the path of the backbone of 10.5 bp per turn of B-DNA (Fig. 3), with a deviation of 2–3 Å towards the 3' ends of each strand which is described below. The positions of the Br atoms indicate that the local axis of the DNA is displaced by a few ångströms from the noncrystallographic 3_1 axis. In fitting the Br positions, we used a smooth supercoil with precise stacking of base pairs at the ends of adjacent 14mers, but the map suggests that a better fit can be obtained with a straight B-DNA model for each 14mer, tilted at $\sim 12^\circ$ with respect to the 3_1 and displaced from it by 4–5 Å, so that the register of the double helix is not maintained from 14mer to 14mer. This model produces nearly perfect overlap of the purine rings of the 5' adenine bases from successive 14mers; the 3' thymines are left unstacked. This exposure may in part account for the deviation of the 3' end of the backbone density from the B-DNA model, and a contact with an R1-69 monomer bound to the adjacent 14mer may also be involved (see below). A perturbation of strict B-DNA structure to fit the density involves a displacement of the 3' end of the 14-base strand by 2–3 Å (Fig. 5). As the 5' end of the opposite strand is not correspondingly displaced, some distortion of normal base-pair geometry may be required to accommodate the backbone shift. The shift seems significant only for the four 3'-terminal bases.

Protein-DNA contacts

As shown in Figs 3, 4 and 5, R1-69 is bound to DNA with $\alpha 3$ inserted in the major groove, a feature anticipated by previous models of repressor-DNA interaction^{1,3,4,8-10,22}. The axis of $\alpha 3$ is displaced by ~ 2 Å from the centre of the major groove. The position of the helix with respect to the base pairs seems to be determined by a set of contacts that the repressor domain makes with the DNA sugar-phosphate backbone (Fig. 5). One of these contacts is near the N-terminus of $\alpha 4$, where a strong bridge connects protein and DNA densities near the position of the phosphate 5' to base +10. (For the numbering of phosphate groups, see Fig. 5.) A less striking bridge occurs near the adjacent phosphate, 5' to base +9. Another strong bridge joins density from the N-terminus of $\alpha 2$ to density from the DNA backbone of the adjacent 14mer, near the phosphate between the two bases at the 3' end of the strand. In phosphate ethylation interference experiments using the 14mer sequence embedded in continuous DNA, Bushman *et al.*² infer that corresponding phosphates are contacted by intact repressor in solution. Ethylation

Table 3 Noncrystallographic symmetry phase refinement¹⁸

Cycle	R	r	Mean phase change
1	0.493	0.436	62.4°
2	0.397	0.618	17.2°
3	0.361	0.688	9.6°
4	0.340	0.732	7.4°
5	0.330	0.751	5.6°
6	0.324	0.761	3.9°
Overall phase change			68.9°

$$R = \sum |F_{\text{obs}} - F_{\text{calc}}| / \sum |F_{\text{obs}}|; \text{ mean phase change} = \sum |\phi_{\text{calc}} - \phi_{\text{obs}}| / N;$$

$$r = \frac{\sum |F_{\text{obs}}| |F_{\text{calc}}| - \sum |F_{\text{obs}}| \sum |F_{\text{calc}}|}{[\sum |F_{\text{obs}}|^2 - (\sum |F_{\text{obs}}|)^2][\sum |F_{\text{calc}}|^2 - (\sum |F_{\text{calc}}|)^2]}.$$

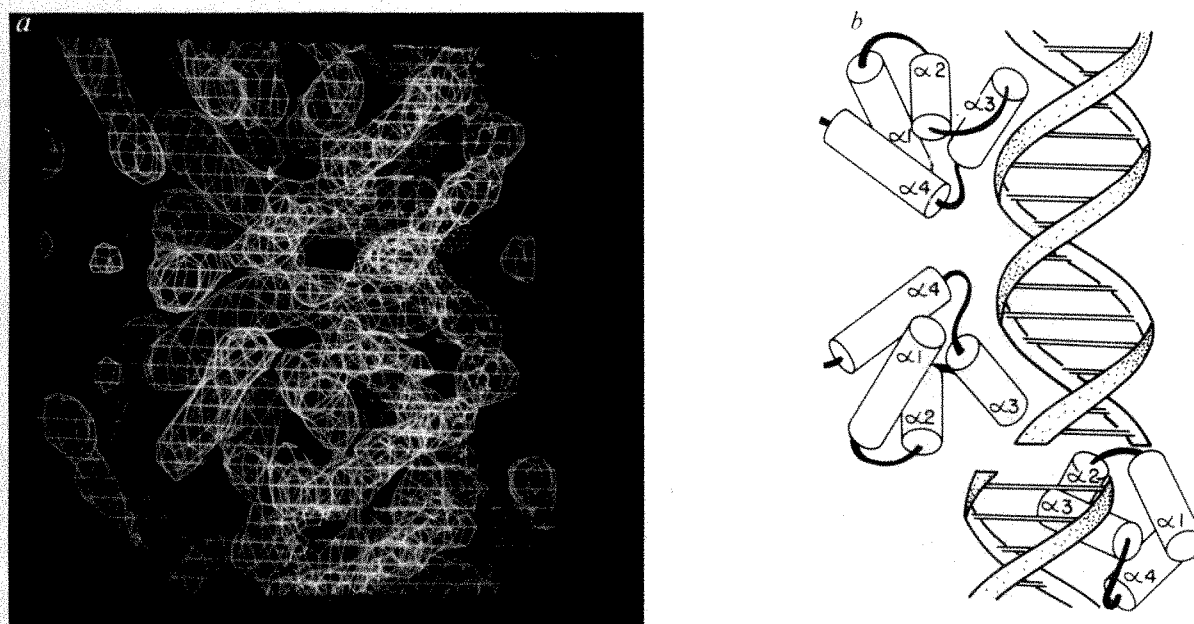


Fig. 3 *a*, Overview of the co-crystal map at 7 Å resolution after six cycles of noncrystallographic symmetry averaging and phase refinement. Almost an entire I422 asymmetric unit, consisting of three R1-69 monomers and one-and-a-half 14mers, is shown. Each protein-DNA complex consists of two protein monomers and one operator. The view is perpendicular both to the noncrystallographic 3_1 and to the dyad of a complex. The positions of Br atoms are shown as stippled spheres. Model B-DNA, tilted and displaced as described in the text, is superimposed on DNA electron density. *b*, Schematic representation of *a*, with α -helices (cylinders) labelled near their N-termini.

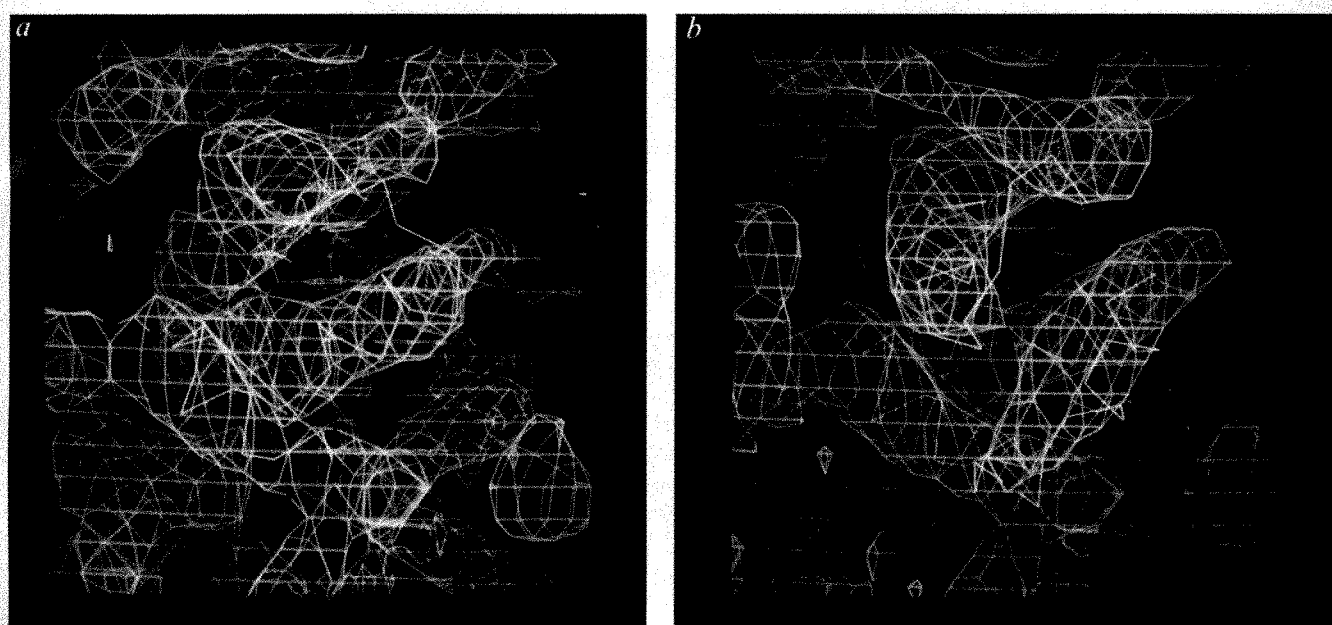
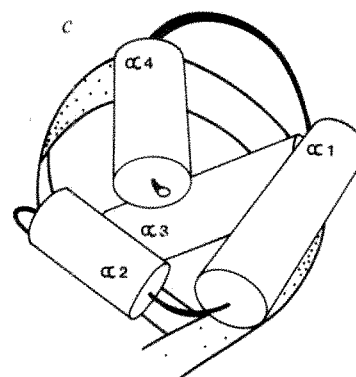


Fig. 4 Structure of R1-69. Straight B-DNA (red) and the α -carbon backbone of λ -repressor residues 17-75 (ref. 1) (gold), including the C-terminal half of $\alpha 1$ and all of $\alpha 2$ - $\alpha 4$, are superimposed on the electron density (see text). *a*, View perpendicular to the noncrystallographic symmetry axis of part of a monomer of R1-69 (the lower one of the dimer in Fig. 3). Helix $\alpha 3$ lies in the major groove of the DNA and $\alpha 2$ lies across $\alpha 3$. A strong bridge of density connects the N-terminus of $\alpha 2$ to the DNA backbone near phosphate +14 (see Fig. 5*b* for phosphate numbering) of the adjacent 14mer. At the top, bridges between protein and DNA are partially visible near phosphates +9 and +10. *b*, View in the same orientation as *a*, but farther from the DNA, showing helices $\alpha 1$ (lower right) and $\alpha 4$ (upper left). Helices $\alpha 2$ and $\alpha 3$ are visible in the background. *c*, Schematic representation of *b*, with the α -helices (cylinders) labelled near their N-termini.



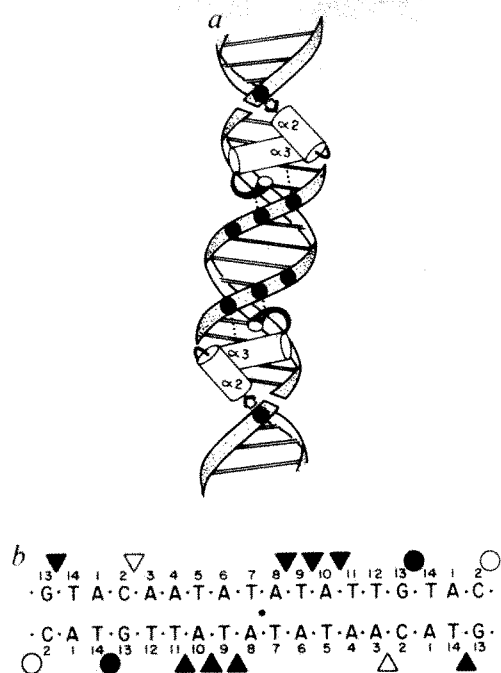


Fig. 5 Protein-DNA backbone contacts. **a**, Schematic drawing showing the contacts. The helix-turn-helix structures and part of the protein backbone just C-terminal to $\alpha 3$ are shown for a dimer of R1-69. The diagram includes one entire 14mer and the ends of the adjacent 14mers, drawn as straight B-DNA. The tilt of the adjacent 14mers is slightly exaggerated. Filled circles indicate positions where the locations of contacts between protein and DNA phosphates suggested by the crystal structure and those inferred from ethylation interference experiments² agree. Dotted lines connect the phosphates to the regions of the protein believed to provide the contacts. The part of the protein responsible for the phosphate contact nearest the centre of the operator is not known. The arrows show the directions of the displacements seen in the crystal of the 3' ends of the DNA strands of the adjacent 14mers (see text). These displacements result in part from contacts made by the N-termini of the $\alpha 2$ helices of the dimer bound to the central 14mer. The corresponding displacements of the 3' ends of the strands of the central 14mer, resulting from proteins bound to the adjacent 14mers, are not shown. **b**, The DNA sequence corresponding to **a**, showing the positions of possible contact between protein and DNA suggested by the crystal structure, and giving the phosphate numbering scheme used in the text. Bases belonging to the same 14mer are connected by small dots. The large dot in the centre locates the 2-fold axis of the central 14mer. Triangles, contacts made by the R1-69 dimer associated with the central DNA 14mer; circles, contacts made by proteins bound to the flanking 14mers. Filled symbols have the same meaning as in **a**; open symbols indicate positions where a contact is suggested by the crystal structure but not detected by ethylation interference².

of the phosphates 5' to bases -1 (the first base outside the 14mer sequence), +9 or +10 on either strand interferes strongly with repressor binding. We believe that the -1 phosphate contact corresponds to the contact in the crystal between $\alpha 2$ and the adjacent 14mer, and that the other two correspond to the bridges of density in the centre of the operator. These results show that the overall configuration of the complex of intact repressor with DNA is similar to that of R1-69, although it is possible that the details of the interactions are slightly different because of constraints imposed by stable dimerization of the intact molecule.

Strictly speaking, confluences of protein and DNA densities such as those just described imply nothing more than close proximity, but it is reasonable to suppose that a bond will form

if the appropriate type of residue is nearby. Groups most likely to interact directly with negatively charged DNA phosphates are those that are positively charged and those that can donate hydrogen bonds. Our confidence that we have correctly identified helices and their connectivity, and how they relate to the amino-acid sequence, permits us to enumerate amino-acid residues that could be involved in these interactions. We suggest that Asn 16 or Gln 17 (or both) might participate in the contact at the N-terminus of $\alpha 2$, Lys 40, Arg 41, or Arg 43 could be involved in the +9 and +10 contacts; some residue C-terminal to $\alpha 4$ might also contact the +9 phosphate. We also note that helices $\alpha 2$, $\alpha 3$ and $\alpha 4$ are arranged so that the N-termini of all three point towards DNA phosphates. The N-terminus of an α -helix carries a virtual partial positive charge¹⁹ which could produce favourable interactions with DNA phosphates to stabilize binding in the observed orientation. A role for interactions between the N-terminal ends of α -helices and backbone phosphates in the binding of λ repressor to DNA has been suggested previously¹.

With the relationship between protein and DNA in our interpretation of the electron density, it is not possible for Asn 16 or Gln 17 to hydrogen bond to the -1 phosphate if the operator is embedded in continuous, straight DNA. If the continuous DNA is given a slight super-twist, however, then both residues are brought to within reasonable hydrogen-bonding distance of the phosphate². This suggests that if the ethylation interference at the -1 phosphate involves disruption of hydrogen bonds donated by Asn 16 or Gln 17, then the DNA is slightly bent (to a radius of curvature of ~ 100 Å) by repressor binding. A slight bend would also enhance any charge interaction that exists between the N-terminus of $\alpha 2$ (see above) and the -1 phosphate. Other deformations of the double helix are possible, but super-twisting (or bending) is the simplest and most easily interpretable. The displacement of 14mers from the noncrystallographic 3₁ axis, as well as the displacement of the 3' end of the DNA strand seen in the crystal (see above and Fig. 5a), may be related to this suggested tendency of repressor to bend DNA when it binds². A monomer of R1-69 bound to a particular 14mer in the crystal can make the $\alpha 2$ contact (which we propose is made to the -1 phosphate on bent continuous DNA in solution) only by lateral displacement of the neighbouring 14mer. The consequently unstacked 3' end of this neighbour might then adjust further to optimize contact with the N-terminus of $\alpha 2$.

There are two other interactions between protein and DNA backbone suggested by our interpretation of the electron density. The N-terminus of $\alpha 3$ closely approaches the DNA backbone, placing the side chain of Ser 30 in a position to hydrogen bond to the phosphate 5' to base +11. Ethylation at that phosphate decreases binding, although less strongly than the modifications described above². Finally, just beyond the C-terminus of $\alpha 3$, an arm of density reaches towards the opposite DNA strand near the phosphate 5' to base +3. The location and orientation of the arm suggest a salt bridge between that phosphate and the side chain of Lys 38, but ethylation at that position has little effect on binding².

Side chains on the external surface of $\alpha 3$ (those that would be exposed to the solvent if not in the complex) are clearly in position to make sequence-specific contacts to the bases in the major groove. The excellent fit of the λ -repressor backbone and of B-DNA to features of the electron density, the invariance in three other structures of the helix-turn-helix motif^{5,20}, and the assignment by sequence similarity of corresponding residues in the λ and 434 repressors^{6,7} permit limited model building of specific interactions between protein and DNA by insertion of 434 repressor side chains onto λ -repressor backbone positioned as in Fig. 5. An important preliminary conclusion is that base pairs 1-4 seem to be accessible to direct interaction with repressor side chains on the $\alpha 3$ helix, with contacts possible between Gln 28 and base pair 1, Gln 29 and base pairs 2 and 3, and Gln 33 and base pairs 3 and 4. In addition, residues in the turn between $\alpha 3$ and $\alpha 4$ can probably contact base pair 5

and possibly base pair 6. We are attempting to test these suggestions and are extending the resolution of the structure determination to 3.2 Å.

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Changing the binding specificity of a repressor by redesigning an α -helix

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We replaced amino acids on the 'outside', or solvent-exposed, surface of the DNA recognition α -helix of 434 repressor with the corresponding amino acids from the recognition helix of P22 repressor. The binding specificity of the resulting hybrid protein, as measured in vivo and in vitro, was that of P22 repressor.

THE X-ray crystal structure of the bacteriophage 434 repressor bound to its DNA operator is described in the accompanying paper¹. One striking feature of the structure is that an α -helix of the protein ($\alpha 3$, or the recognition helix) lies in the major groove of the B-form operator DNA where it presumably makes sequence-specific contacts. This helix is part of a conserved 'helix-turn-helix' unit found in the structures of three other sequence-specific DNA-binding proteins (λ repressor, λ cro protein, and *Escherichia coli* catabolite gene activator protein (CAP))²⁻⁶. For each of these proteins, model building has suggested that the recognition helix can be placed in the major groove of the relevant operator⁷⁻⁹. The conserved helix-turn-helix unit has been predicted to exist in many other DNA-binding proteins based on amino-acid sequence homologies¹⁰⁻¹². Analyses of mutant proteins¹³⁻¹⁸ are consistent with the proposal that at least part of the specificity of binding is determined by the recognition helix in each of several cases.

In the experiments described here, we have redesigned the recognition helix of 434 repressor so that the resulting protein has a new biological activity. On the outside (solvent-exposed) surface of the α -helix, the redesigned 434 repressor bears amino acids that are presumed to lie at the corresponding positions on the recognition helix of a different repressor, that encoded by the *Salmonella* bacteriophage P22. Amino acids on the 'inside' surface of the helix were unchanged. We reasoned that this strategy would allow the new amino-acid sequence to fold into an α -helix in the context of the remainder of the protein. *In vivo*, the parental 434 repressor binds only to 434 operators, but the redesigned 434 hybrid repressor, named 434R [$\alpha 3$ (P22R)], binds only to P22 operators. *In vitro*, purified 434R [$\alpha 3$ (P22R)] binds specifically to P22 operator DNA. Furthermore, it binds to the three binding sites in wild-type P22 O_R with the same affinity order as does P22 repressor, namely $O_{R1} = O_{R2} > O_{R3}$. The apparent affinities of P22 repressor and 434R [$\alpha 3$ (P22R)] for P22 operator DNA are comparable. We conclude first, that in 434 repressor, amino acids along the recognition helix are solely responsible for distinguishing between binding to two different sets of operators; and second, 434 repressor and P22

repressor present their recognition helices in very similar ways to 434 operators and P22 operators, respectively.

Redesigning the recognition helix

The presumptive configurations of the recognition helices and flanking residues of the 434 and P22 repressors are shown in Fig. 1a. The arguments that led to the assignment of these particular configurations are summarized in Fig. 1 legend. To redesign 434 repressor, we assumed that residues on the inside surface of its helix, which pack against the body of the repressor, could not be substituted without destabilizing the protein. We also assumed that amino acids on the outside surface, which are free to interact with DNA in the protein-DNA complex, could be substituted without strongly affecting the stability of the protein. Figure 1b shows the presumptive configuration of the redesigned recognition helix and flanking residues of 434R [$\alpha 3$ (P22R)]. Compared with wild-type 434 repressor, 434R [$\alpha 3$ (P22R)] bears five amino-acid substitutions taken from P22 repressor.

Figure 1c shows the sequence of a fragment of the gene encoding 434R [$\alpha 3$ (P22R)], and, schematically, the strategy by which it was constructed. Using mismatched primer mutagenesis *in vitro*, we introduced restriction enzyme cleavage sites that bracket the region encoding the recognition helix in the 434 repressor gene. This region was then removed from the 434 repressor gene by cleavage with the appropriate restriction enzymes, and replaced with a new DNA sequence, constructed from four synthetic oligonucleotides, which encodes the hybrid helix of 434R [$\alpha 3$ (P22R)]. The hybrid gene was fused to the *lac* promoter to create the plasmid pRW219. When pRW219 was introduced into an appropriate *E. coli* strain, ~1% of cellular protein was 434R [$\alpha 3$ (P22R)] (data not shown).

434R [$\alpha 3$ (P22R)] activity *in vivo*

E. coli cells that are producing the repressor encoded by a particular phage are immune to infection by that phage. Because P22 is a *Salmonella* phage which does not grow in *E. coli* strains, hybrid phages named λimm^{434} and λimm^{P22} were used in the

Fig. 1 Redesigning the outside surface of the recognition helix of 434 repressor. *a*, Schematic representations of the presumptive recognition helices of the 434 and P22 repressors. *b*, Two schematic representations of the presumptive configuration of the recognition helix of 434R [$\alpha 3$ (P22R)]. *c*, Construction of the gene encoding 434R [$\alpha 3$ (P22R)]. *a*, The dispositions of amino acids were assigned by superimposing the conserved helix-turn-helix units predicted to exist in the 434 and P22 repressors¹⁰ on the helix-turn-helix unit seen in the X-ray crystal structure of λ repressor². We have previously presented results that support this disposition of amino acids in the recognition helix of 434 repressor¹³, and Anderson *et al.*¹ have recently demonstrated the existence in 434 repressor of a helix-turn-helix unit. The Gly residue, present at the turn in almost all predicted helix-turn-helix units¹⁵, is triply underlined for reference. The amino-acid sequence shown for P22 repressor is actually that of the *pcA* mutant P22 repressor³⁰, in which Lys replaces the Glu of wild-type P22 repressor at residue 42. Residue 42 is the underlined lysine in the following sequence in the figure: ... Ser-Lys-Thr-Glu ... No differences between the DNA-binding activities of wild-type P22 repressor and *pcA* mutant P22 repressor have been observed *in vivo*³⁰ or in an extensive series of measurements *in vitro* (J. Douhan III and M.P., unpublished data; see also Fig. 3). Therefore, in constructing the gene encoding 434R [$\alpha 3$ (P22R)], we chose not to change the residue at the corresponding position in 434 repressor, Lys 38. *b*, Amino acids taken from P22 repressor are drawn in white letters inside black boxes. The dashed lines at either end of the first drawing indicate that this helix is embedded in the remainder of the 434 repressor protein. The second representation³¹, a view from the carboxy-terminal end of the helix in projection down the helical axis, emphasizes the spatial segregation of P22 repressor-derived amino acids on the outside surface of the helix (white letters in black boxes) and 434 repressor-derived amino acids on the inside surface of the helix (black letters). Note that the amino-acid substitution at position -1 of the recognition helix is not present in this second representation and that the recognition helices of 434 repressor and P22 repressor have common amino acids at positions 4, 6 and 8. *c*, A portion of the gene encoding 434R [$\alpha 3$ (P22R)] is shown with the corresponding amino-acid sequence. The conserved glycine residue is again triply underlined for reference (see *a*). The five amino-acid substitutions in 434R [$\alpha 3$ (P22R)] are marked by arrows. DNA to the left of the *KpnI* site and to the right of the *DdeI* site encodes the 193 amino acids that are identical in 434 repressor and 434R [$\alpha 3$ (P22R)]. DNA between the *KpnI* and *DdeI* sites was assembled from four synthetic oligonucleotides which are represented by two white and two stippled boxes. Also shown are sites for the restriction enzymes *MboI* and *TaqI* which were used in the experiments described in Fig. 4. These sites are common to the genes encoding 434 repressor and 434R [$\alpha 3$ (P22R)].

Methods. A site for the enzyme *KpnI* was introduced into the gene encoding 434 repressor to create the plasmid pRW199. We used an oligonucleotide with the sequence: 5'-TGCTGGGTGTACCCACCTT-3' which was mismatched with bases in the wild-type gene at the two underlined nucleotides. Mutagenesis was performed as described previously¹³ using the methods of Zoller and Smith³² with the following exceptions: single-stranded DNA was prepared from the *dam*⁻ *E. coli* strain RW102 (our unpublished data), and the template DNA was a derivative of the plasmid, pEMBL8+ (ref. 33). The four synthetic oligonucleotides shown in *c* which encode part of 434R [$\alpha 3$ (P22R)] were assembled essentially as described by Sgarbetta and Khorana³⁴, and the resulting 39-bp duplex which had *KpnI*- and *DdeI*-compatible ends was isolated by gel electrophoresis. This DNA fragment was substituted for the corresponding 39-bp fragment of pRW199 essentially as described previously¹³.

Table 1 Immunity specificity of 434R [$\alpha 3$ (P22R)] *in vivo*

Protein	Immunity to	
	λimm^{434}	λimm^{P22}
434 R	+	-
P22 R	-	+
434 R [$\alpha 3$ (P22 R)]	-	+

Phages were spotted lawns of three host strains, each of which was producing one of the repressors listed in the column on the left. Following overnight incubation, the lawns were examined for evidence of plaque formation and host killing. + Indicates that host cells were immune to infection (no apparent cell lysis) and - indicates the converse. Identical results were obtained following overnight growth at either 30 or 37 °C. λimm^{434} and λimm^{P22} are hybrid phages in which the repressor gene and operators of phage λ are replaced by the repressor gene and operators of phages 434 and P22, respectively^{19,20}. These phages behave with regard to their immunity properties exactly as do the parental phages, 434 and P22^{19,20}. In all cases where phage growth occurred, phage spots were turbid except when λimm^{434} was spotted on cells producing 434R [$\alpha 3$ (P22R)]. Each of the three proteins in the table was at least 1% of cellular protein. The bacterial host strains for these experiments were derivatives of XA90 (ref. 26) which carried an F'(*lac*^{Q1}) episome²⁷ as well as the plasmids listed below. Spot tests were performed in the presence of 1 mM isopropyl β -D-thiogalactopyranoside (IPTG) to induce the synthesis of each of the repressors whose genes are transcribed by *lac* operon promoters. Listed below for each repressor is the name of the plasmid that was introduced into XA90 cells to direct synthesis of the protein, and the estimate of the percentage of total protein that consisted of repressor in these cells based on electrophoresis in SDS-polyacrylamide gels²⁸ of total cellular protein: 434 repressor, pRW88 (ref. 13), 1% (or pRW190, 10%, our unpublished data); P22 repressor, pTP15 (ref. 29), 1%; 434R [$\alpha 3$ (P22R)], pRW 219 (see Fig. 1), 1%.

experiments described below. In these hybrid phages, the repressor gene and operators of phage λ have been replaced by the repressor gene and operators of phages 434 and P22, respectively^{19,20}. *In vivo*, the DNA-binding activity of 434 repressor or P22 repressor is extremely specific: cells producing 434 repressor are immune to infection by λimm^{434} phages but not immune to infection by λimm^{P22} phages¹⁹, whereas cells producing P22 repressor are immune to infection only by λimm^{P22} phages²⁰. This binding specificity is maintained even when cells are producing very large quantities of either 434 or P22 repressor (see Table 1). (For reference, Fig. 2 shows consensus DNA sequences for 434 and P22 operators.)

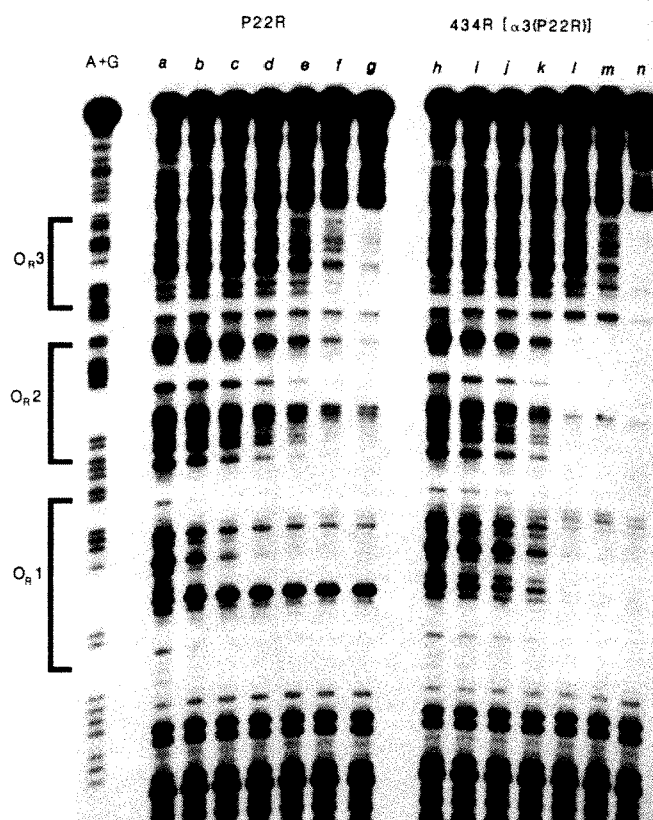
To test the DNA-binding specificity of the hybrid repressor, 434R [$\alpha 3$ (P22R)], we spotted λimm^{434} phages and λimm^{P22} phages on a lawn of cells producing 434R [$\alpha 3$ (P22R)]. These cells were immune to infection by the λimm^{P22} phages and not immune to infection by the λimm^{434} phages (Table 1). The degree of immunity conferred was indistinguishable from that conferred by wild-type P22 repressor: when 10^8 λimm^{P22} phages were spotted on a lawn of cells producing 434R [$\alpha 3$ (P22R)],

A . T . A A G C T T . A . T P22 Operator
A C A A T T G T 434 Operator

Fig. 2 Consensus sequences for P22 and 434 operators. Shown are consensus sequences along one DNA strand for P22 and 434 operators^{13,23}, aligned at their centres of symmetry which are indicated by ●. An × in either sequence indicates a non-conserved base at that position of the operator.

Fig. 3 Binding of 434R [$\alpha 3$ (P22R)] to P22 O_R *in vitro*. Autoradiogram resulting from a typical DNase I protection experiment in which purified P22 repressor or 434R [$\alpha 3$ (P22R)] was bound to DNA containing wild-type P22 O_R . End-labelled DNA fragments in the lane marked A + G were cleaved at purines to generate size markers. The boundaries of each 18-bp binding site (O_{R1} , O_{R2} , and O_{R3}) are indicated on the left of the figure. Lanes a–g represent the electrophoresis of binding reactions that contained successively higher concentrations of P22 repressor. Lanes h–n represent the electrophoresis of binding reactions that contained successively higher concentrations of 434R [$\alpha 3$ (P22R)]. In all the reactions, the concentration of O_R -containing DNA fragments was $\sim 10^{-10}$ M. The concentrations of active protein monomers in each binding reaction were as follows: a, 0; b, 7.6×10^{-10} M; c, 1.3×10^{-9} M; d, 2.2×10^{-9} M; e, 3.7×10^{-9} M; f, 6.2×10^{-9} M; g, 1.1×10^{-8} M P22 repressor; and h, 0; i, 5.2×10^{-9} M; j, 8.8×10^{-9} M; k, 1.5×10^{-8} M; l, 2.5×10^{-8} M; m, 7.0×10^{-8} M; n, 5.6×10^{-7} M 434R [$\alpha 3$ (P22R)]. At saturating concentrations of each protein, both P22 repressor and 434R [$\alpha 3$ (P22R)] protect from DNase I cleavage regions of equivalent size, even though the precise boundary of the protected region below O_{R1} is not readily apparent because DNase I cleaves only weakly in this region. Examination of over-exposed autoradiograms reveals that the region protected by both proteins extends to the G residue 5 bp outside O_{R1} on the labelled strand. We note that a single phosphodiester bond within O_{R1} is not protected from DNase I cleavage by binding of P22 repressor although it is protected by 434R [$\alpha 3$ (P22R)]. (This phenomenon has been observed previously²⁴.) Using a DNA fragment labelled on the other strand, we observed that all of the phosphodiester bonds within O_{R1} were protected by each protein. The protein used in the experiment shown in the figure was *pcA* P22 repressor (see Fig. 1 legend). We have observed no significant differences between the binding of wild-type P22 repressor and *pcA* P22 repressor to O_R DNA. A detailed analysis of the binding of wild-type P22 repressor to O_R has been reported by Poteete and Ptashne²⁴. In the experiment shown here, we observed half-maximal occupancy of O_{R1} at a concentration of 1.0×10^{-9} M P22 repressor and at 8.8×10^{-9} M 434R [$\alpha 3$ (P22R)]. In nitrocellulose filter-binding experiments³⁵, the concentrations of 434R [$\alpha 3$ (P22R)] and P22 repressor monomers required to retain on the filter one-half of the DNA fragments in binding reactions were 1.6×10^{-10} M and 3.5×10^{-9} M, respectively (data not shown). In these experiments, the concentration of DNA fragments was 5×10^{-12} M, and the binding reaction conditions were slightly different (see methods). The concentrations of P22 *pcA* repressor required for half-maximal operator occupancy that we have determined in these experiments are virtually identical to those determined earlier for wild-type P22 repressor^{22,24}. From DNase I protection experiments similar to that shown here, we deduced the following ratios of protein concentrations required for half-maximal occupancy of O_{R1} , O_{R2} and O_{R3} on a DNA fragment-bearing wild-type O_R : for P22 *pcA* repressor, 1:1.5:6; and for 434R [$\alpha 3$ (P22R)], 1:1:6. The affinity order of P22 *pcA* repressor is identical to that determined earlier for wild-type P22 repressor²⁴. In the absence of cooperative interactions, threefold higher concentrations of P22 *pcA* repressor or of 434R [$\alpha 3$ (P22R)] were required to occupy O_{R3} half-maximally than were required to occupy O_{R1} half-maximally in DNase I protection experiments in which each site was present on a separate DNA fragment (data not shown). For each of three proteins, we list below the highest concentration tested at which we did not detect specific binding to non-cognate operator DNA and the ratio of this value over the concentration at which specific binding to cognate operator DNA was detectable: 434 repressor, 4×10^{-6} M, 100; P22 *pcA* repressor, 2×10^{-7} M, 300; 434R [$\alpha 3$ (P22R)], 1×10^{-6} M, 100.

Methods. 434R [$\alpha 3$ (P22R)] was purified from cells containing pRW219 following the same protocol by which 434 repressor has been purified³⁶. We measured the stability of 434R [$\alpha 3$ (P22R)] by digesting it with the thermo-stable protease thermolysin at various temperatures¹⁴. The amino-terminal domain of wild-type 434 repressor was stable to digestion at temperatures as high as 60°C whereas the amino-terminal domain of 434R [$\alpha 3$ (P22R)] was stable to digestion only at temperatures as high as 37°C (data not shown). We have not investigated further this apparent instability of 434R [$\alpha 3$ (P22R)] *in vitro*. Three different end-labelled DNA fragments were used in the binding experiments described in the figure: a *HindIII*/*CfoI* fragment from pTP37 (ref. 24) which contains wild-type P22 O_R ; an *EcoRI*/*HindIII* fragment from pRW253 which contains O_{R1} ; and an *EcoRI*/*HindIII* fragment from pRW254 which contains O_{R3} . (The asterisk indicates the labelled end of each fragment.) pRW253 and pRW254 were generated by partially cleaving a fragment of pTP37 with *HinfI* (which cleaves within O_{R2}), and then separately cloning the appropriate O_{R1} - and O_{R3} -containing DNA fragments into pEMBL8+. The reaction conditions for DNase I protection experiments were: 10 mM HEPES (pH 7.0), 200 mM KCl, 1 mM $MgCl_2$, 0.5 mM $CaCl_2$, 100 $\mu g ml^{-1}$ sonicated chick blood DNA at 23°C. The reaction conditions for the nitrocellulose filter binding experiment described in the legend were identical except that the divalent cations and chick blood DNA were omitted.



to lysis of the cells was apparent. Also, production of 434R [$\alpha 3$ (P22R)] had no detectable effect on the number or size of plaques formed by λimm^{434} phages (see Table 1). Thus, the five amino-acid substitutions introduced into 434 repressor to create 434R [$\alpha 3$ (P22R)] have switched the biological specificity of the protein from 434 operator binding to P22 operator binding.

Another observation from these physiological tests supports our conclusion that 434R [$\alpha 3$ (P22R)] has a negligible affinity for 434 operators. Wild-type λimm^{434} phages (encoding a functional repressor) form turbid plaques when spotted on lawns of either wild-type cells or cells producing P22 repressor, whereas mutant λimm^{434} phages (encoding a defective repressor) form clear plaques on the same lawns. We found that wild-type λimm^{434} phages, when spotted on a lawn of cells producing 434R [$\alpha 3$ (P22R)], formed clear rather than turbid plaques (see Table 1). We imagine that this interference with the function of the repressor encoded by the infecting λimm^{434} phages is a result of the fact that heterodimers of 434 repressor and 434R [$\alpha 3$ (P22R)] do not bind to 434 operators. We know that 434 repressor, which binds as a dimer, is a two-domain protein in which the amino-terminal domain mediates DNA binding

and the carboxy-terminal domain mediates dimerization^{13,21}. As 434R [$\alpha 3$ (P22R)] and wild-type 434 repressor have the same carboxy-terminal domains, they presumably form heterodimers efficiently. In the presence of excess 434R [$\alpha 3$ (P22R)], virtually all of the 434 repressor in cells infected with wild-type λimm^{434} phages would be sequestered in the form of mixed dimers; these mixed dimers evidently do not bind with significant affinity to 434 operator DNA.

434R [$\alpha 3$ (P22R)] activity *in vitro*

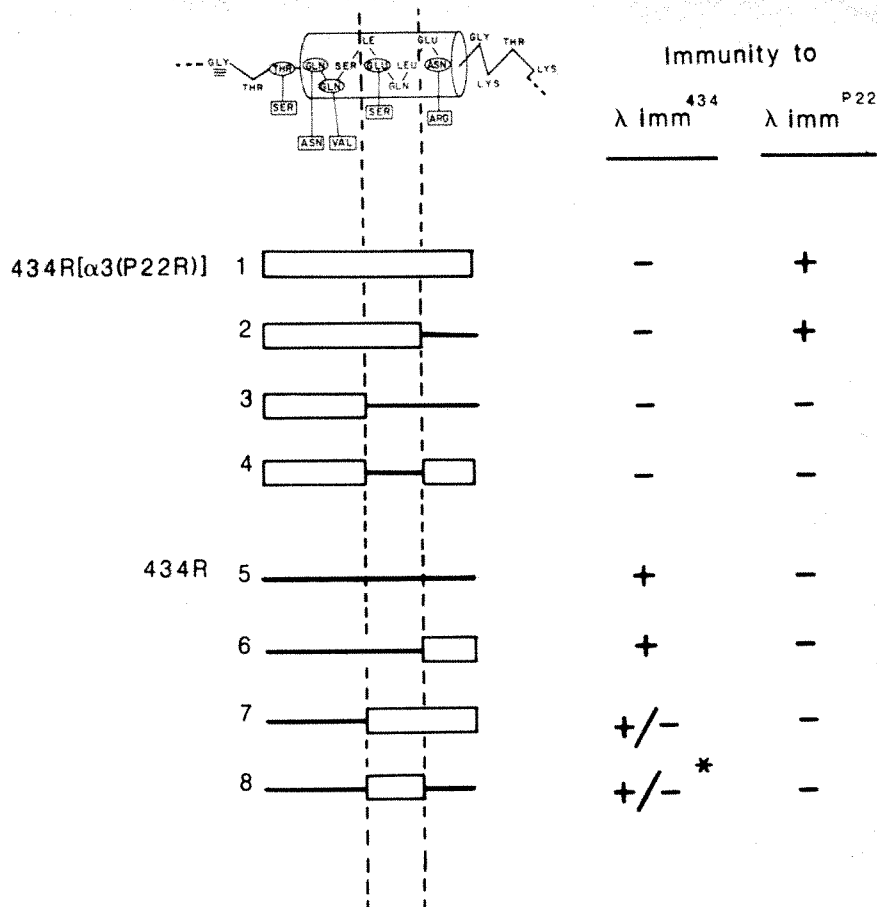
Purified 434R [$\alpha 3$ (P22R)] bound specifically to DNA containing the right operator (O_R) of phage P22 in DNase I protection experiments (Fig. 3). P22 repressor and 434R [$\alpha 3$ (P22R)] identically protected the same regions of O_R -containing DNA from DNase I cleavage with one exception: P22 repressor did not protect a single phosphodiester bond within O_{R1} that is protected by binding of 434R [$\alpha 3$ (P22R)] (see Fig. 3). As expected, based on the operator specificity exhibited by 434R [$\alpha 3$ (P22R)] *in vivo* (see Table 1), we failed to observe specific binding of 434R [$\alpha 3$ (P22R)] (or P22 repressor) to 434 operator DNA even at high repressor concentrations (see Fig. 3 legend). Similarly, we failed to observe specific binding of 434

Fig. 4 Activity *in vivo* of recombinants between 434 repressor and 434R [$\alpha 3$ (P22R)]. The diagram on the left indicates schematically the identity of the amino acids in the recognition helix of each protein. P22 repressor-derived amino acids from 434R [$\alpha 3$ (P22R)] are indicated by open boxes and 434 repressor-derived amino acids by solid lines. For reference, we show a drawing of the recognition helix of 434 repressor in which each of the five amino acids that were substituted in creating 434R [$\alpha 3$ (P22R)] is circled and connected to a box containing the amino-acid substitution. Dashed vertical lines indicate the boundaries within the recognition helices of the three groups referred to in the text (A, B and C). As in Table 1, + indicates that a bacterial strain producing a particular protein was immune to infection (no apparent cell lysis) and - indicates the converse. In lines 7 and 8, +/- indicates that strains producing these proteins were partially immune by the following criteria: wild-type λ imm⁴³⁴ phages formed fewer, smaller plaques than they did on strains producing no repressor, and λ imm⁴³⁴ c (clear) phages formed fewer, smaller and more turbid plaques than they did on strains producing no repressor (data not shown). This turbidity presumably is caused by the growth of partially immune microcolonies in the centres of phage spots. Each of the proteins, except that of line 8, was tested under conditions where it constituted ~1% of total cellular protein as determined by examination of stained bands following gel electrophoresis of whole-cell lysates under denaturing conditions (unpublished data). The asterisk in line 8 indicates that production of high levels of this protein prevented the growth of cells. Therefore, the immunity conferred by this protein was tested under conditions where it constituted <1% of cellular protein.

Methods. Genes encoding the recombinants shown in lines 3 and 7 were generated by crossing the genes encoding 434 repressor and 434R [$\alpha 3$ (P22R)] at the *Mbo*I restriction enzyme cleavage sites common to the DNA fragments encoding their recognition helices (see Fig. 1c). Then the genes encoding the recombinants shown in lines 2, 4, 6 and 8 were generated by crossing the appropriate combinations of genes encoding the proteins shown in lines 1, 3, 5 and 7 at the *Taq*I restriction enzyme cleavage sites common to the DNA fragments encoding their recognition helices. In the DNA sequence shown in Fig. 1c, another site for the enzyme *Taq*I exists, but cleavage at this site is blocked by methylation *in vivo* at the overlapping site for the enzyme *Mbo*I. All the recombinant genes were ligated into a common backbone from the plasmid pRW220, and the resulting plasmids were then transformed into XA90(F' *lac*^{O1}) cells for immunity tests (see Table 1). pRW220 is a derivative of pRW88 (ref. 13) in which the 248-bp *Eco*RI/*Hind*III fragment was replaced by the ~75-bp *Eco*RI/*Hind*III fragment from the plasmid π VX (ref. 37). The DNA sequence of the relevant portion of each of the genes was confirmed by the chain termination method³⁸. The level of each protein *in vivo* was estimated as described in Table 1.

repressor to P22 operator DNA *in vitro*. Comparable concentrations of either P22 repressor or 434R [$\alpha 3$ (P22R)] monomers were required to occupy *O_R* DNA half-maximally in several different DNA-binding experiments (see Fig. 3 legend). We cannot assign precise values for the affinity of the dimer of either protein as we do not know the value of the equilibrium constant for the dimerization reaction of either protein. Dimerization of P22 repressor and of 434 repressor (and therefore presumably also of 434R [$\alpha 3$ (P22R)]) is mediated in each case mainly by association of carboxy-terminal domains^{21,22}; these domains are different in the two proteins.

The experiment shown in Fig. 3 also demonstrates that a more subtle characteristic of the binding of 434R [$\alpha 3$ (P22R)] to P22 operators was similar to that of P22 repressor. In *O_R*, there are three similar but not identical 18-base-pair (bp) sites (named *O_R1*, *O_R2* and *O_R3*) (ref. 23), and P22 repressor binds to these sites on a DNA molecule bearing wild-type *O_R* with the affinity order *O_R1* ≈ *O_R2* > *O_R3* (ref. 24). As shown in Fig. 3, 434R [$\alpha 3$ (P22R)] bound with the same affinity order as did P22 repressor. Both P22 repressor and 434 repressor (and therefore presumably also 434R [$\alpha 3$ (P22R)]) bind cooperatively to adjacent operator sites on a single DNA molecule, and this cooperativity is mediated by the carboxy-terminal domain of each protein^{13,21,22,24}. We also measured the relative affinities of each protein for *O_R1* and *O_R3* in the absence of cooperative interactions. In DNase I protection experiments with *O_R1* and *O_R3* on two different DNA molecules, P22 repressor and 434R [$\alpha 3$ (P22R)] each bound with higher affinity to *O_R1* than to *O_R3* (see Fig. 3 legend).



Subdividing helix 3

We wished to determine whether all of the five amino-acid substitutions introduced in creating 434R [$\alpha 3$ (P22R)] were required for the ability of the protein to bind to P22 operators. Therefore, we took advantage of restriction enzyme cleavage sites present inside the regions encoding the recognition helices of 434 repressor and 434R [$\alpha 3$ (P22R)] (see Fig. 1c). We divided the five amino-acid substitutions into three groups (referred to below as groups A, B and C) and studied the activity *in vivo* of the various recombinants shown in Fig. 4. Group A consists of the three amino-acid substitutions at positions -1, 1 and 2 in the recognition helix; group B consists of the single amino-acid substitution at position 5; and group C consists of the single amino-acid substitution at position 9. All of the proteins referred to in Fig. 4 were stable *in vivo* by the following criterion: when the *lac* promoter driving expression of each protein was fully derepressed, each protein constituted ~1% of cellular protein (data not shown).

We find that, first, the P22 repressor-derived amino acids of groups A and B were necessary and sufficient to determine P22-operator binding specificity. Because we have not attempted to separate the three amino acids in group A, we cannot specify the minimum number of amino-acid substitutions required for P22 operator-binding activity. Second, the 434 repressor-derived amino acids of group A were sufficient for detectable binding to 434 operators, but for full activity (comparable to that of wild-type 434 repressor), the 434 repressor-derived amino acids of both groups A and B were necessary. Third, for all of the

proteins in Fig. 4, the identity of the amino acid in group C (either Asn or Arg) had no detectable effect on the binding activity. Fourth, none of the proteins simultaneously recognized 434 operators and P22 operators.

We also observed that cells producing a 434 repressor protein bearing a single amino-acid substitution, the P22 repressor-derived serine of group B, exhibited the following peculiar property: fewer than 1 in 10^6 cells survived to form colonies when the *lac* promoter driving expression of this protein was fully derepressed. We speculate that the extreme toxicity of this mutant protein may be caused by a drastically enhanced affinity for nonspecific DNA. This phenotype has been observed by Nelson and Sauer²⁵ for a mutant λ repressor that bears a substitution of Ser for Gly at position 5 of its recognition helix.

Conclusions

The experiments reported here extend our earlier work in which we studied a hybrid 434 repressor bearing the recognition helix of 434 cro protein¹³. 434 repressor and cro protein have very similar DNA-binding activities: they each bind with high affinity to the same set of 434 operators. In addition, the amino-acid sequences of the two proteins are very similar in their DNA-binding domains¹⁰, and it was therefore possible to exchange directly the recognition helix of 434 cro protein for that of 434 repressor without otherwise altering either protein. The resulting hybrid repressor was stable and active, presumably because the amino-acid residues on the inside surfaces of the recognition helices of both proteins are nearly identical¹³.

In the experiments reported here, we studied a 434 repressor hybrid that bears the recognition helix of a more distantly related protein. 434 repressor and P22 repressor have different DNA-binding activities. Even at very high concentrations, neither protein binds detectably to non-cognate operators either *in vivo* or *in vitro*. The amino-acid sequences of the two proteins are sufficiently dissimilar that it seemed unlikely that simply substituting the P22 repressor helix for that of 434 repressor would have yielded a stable and functional protein. We therefore engineered the 434 repressor recognition helix, replacing amino acids on its outside surface with the appropriate amino acids of P22 repressor, and leaving amino acids on its inside surface

as 434 repressor amino acids to preserve the structure of the folded protein. The resulting protein bound specifically and with high affinity to P22 operators *in vivo* and *in vitro*. We conclude that the recognition helix of 434 repressor is the sole determinant in 434 repressor that distinguishes between two different specificities: binding to 434 operators or to P22 operators. Some part of P22 repressor other than its recognition helix may also make sequence-specific DNA contacts, but our results require that 434R [$\alpha 3$ (P22R)] make these contacts as well.

As the properties of P22 repressor and 434R [$\alpha 3$ (P22R)] are so similar, we further conclude that the recognition helices of 434 repressor and P22 repressor interact with their respective operators in very similar ways. This conclusion is contrary to other proposals for the recognition helices of λ repressor⁷, λ cro protein⁸ and *E. coli* CAP⁹. Model-building studies of possible structures of the protein-DNA complexes formed by these three proteins suggest that each of the three recognition helices interacts with its operator in a significantly different manner.

Our results are consistent with what has been learned from studying the structure of a 434 repressor-operator complex. Anderson *et al.*¹ have observed that α -helix 3 of 434 repressor (its recognition helix) lies in the major groove with its amino-terminal end in intimate contact with the surface of the operator. Our analysis of recombinants between 434 repressor and 434R [$\alpha 3$ (P22R)] suggests that the amino-terminal end of the recognition helix is more important in determining the binding specificity of the protein than is the carboxy-terminal end. Similar suggestions have been made for λ repressor² and *E. coli* CAP⁹ based on model building, and a recent report on the effects of amino-acid substitutions in the recognition helix of λ cro protein is also consistent with this idea¹⁸. Further experiments will be required to understand in detail the roles of individual amino acids on the outside surface of the recognition helix in operator binding.

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Reconstitution of an active surface T3/T-cell antigen receptor by DNA transfer

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We have introduced a full-length complementary DNA of the T-cell antigen receptor β -chain into a mutant human T-cell line that lacked a complete β -chain messenger RNA, had a diminished level of α -chain transcript and did not express surface T3- or antigen receptor. Expression of the transfected β -chain led to a normal level of α -chain transcript and a structurally and functionally active T3 T-cell antigen receptor complex on the cell surface.

THE T-cell antigen receptor that functions to recognize foreign antigens is a disulphide-linked heterodimer of relative molecular mass (M_r) 80,000–90,000 (80–90 K), composed of an acidic α - and a basic β -chain^{1–3}. The human^{4–6} and murine^{7–9} cDNAs have recently been cloned, shown to undergo somatic rearrangements in T cells^{4–9} and are related^{4–9} to, but distinct^{10–12} from, immunoglobulin genes. The receptor heterodimer is associated on the cell surface with T3, a set of three non-covalently linked peptides of 20–28 K^{13–17}. In an attempt structurally and functionally to separate the antigen receptor and T3, we selected for mutants of the T-cell tumour line Jurkat, which lacked either T3 or the T-cell antigen receptor, using OKT3 or an antiidiotypic antibody (C305). However, all mutants, selected either way, failed to express both these molecules¹⁶ on the surface. We show here that three mutants that do not express a full length β -mRNA have diminished levels of α -chain specific RNA, and do not express surface T3. Transfection of one of the mutants with β -chain complementary DNA induced the expression of normal levels of α -chain mRNA and led to the expression of a functional T3/T-cell antigen receptor complex on the cell surface.

Intracellular T3 mRNA and proteins

The three subunits of T3 consist of a 26–28 K γ -chain and two chains of 20–30 K, one a glycosylated δ -chain and the other a non-glycosylated ϵ -chain^{18–20}. Using a cDNA probe to the T3 δ -chain (pGBC-9)²¹, we examined seven T3/T-cell antigen receptor-negative mutants of Jurkat derived from four separate mutagenizations¹⁶ for the presence of transcripts. A Northern blot²² obtained with RNA isolated from four of these mutants, the wild-type cell (Jurkat) and HPB-ALL (Fig. 1a), shows that all mutants examined make transcripts for the T3 δ -chain that are indistinguishable in size from the normal T3- δ transcript.

We also examined wild-type Jurkat and the mutants for intracellular T3 subunits by biosynthetic labelling with ³⁵S-methionine and ³⁵S-cysteine. Several hours of continuous labelling, immunoprecipitation and SDS polyacrylamide gel electrophoresis revealed that all mutants had a doublet at 22–23 K precipitated by anti-Leu 4 from the mutant that is indistinguishable from that detected in Jurkat (Fig. 1b). During shorter labelling periods (15 min), the γ -chain of T3 was also detectable with anti-Leu 4 antibody in all the mutants (data not shown). Endoglycosidase F digestion of the Leu 4 immunoprecipitates from all three cell lines resulted in the loss of only the upper band of the 22–23 K doublet and the appearance of two lower M_r bands of 15 and 17 K (data not shown). These results are consistent with the notion that the upper band of the doublet represents the glycosylated T3 δ -chain and the lower band the non-glycosylated T3 ϵ -chain, as described by others^{18,19,23}.

Either α - or β -chain mRNA in mutants

We examined the cells for transcription of the α - and β -chain genes. Figure 2a is a Northern blot of poly(A)-enriched RNA from Jurkat, along with seven T3 T-cell antigen receptor negative mutants, hybridized to a full-length β -chain probe. Jurkat expresses both a 1.3- and a 1.0-kilobase (kb) β -chain transcript^{24–26}; the 1.3-kb transcript of Jurkat represents a full-length completely rearranged gene containing $V_\beta 1D_\beta 1J_\beta 1C_\beta 1$ (V , variable; D , diversity; J , joining; C , constant regions), whereas the 1.0-kb transcript contains only a $J_\beta 2C_\beta 2$ gene segment²⁷. In three of the seven mutants examined, the 1.3-kb transcript was not detected, although the 1.0-kb transcript was always present (Fig. 2a, lanes 2, 4 and 7). In contrast, the other four mutants contained both 1.3- and the 1.0-kb transcripts that could not be distinguished by size from those detected in the wild-type Jurkat cells (Fig. 2a, compare lane 1 with lanes 3, 5, 6 and 8).

Figure 2b shows that Jurkat also contains a 1.5-kb transcript homologous to the α -chain cDNA probe (lane 1). Four mutants seem to contain greatly diminished levels of α -chain transcripts (lanes 2, 3, 4 and 7). Interestingly, three of these mutants lack the 1.3-kb β -chain transcript (lanes 2, 4 and 7). The remaining three mutants seemed to contain normal levels of both α - and β -chain mRNA.

Transfection with β -chain cDNA

We next investigated the effect of transferring cDNA encoding the β -chain into a mutant that does not express the full-length of 1.3-kb mRNA. We used the Friend spleen focus-forming virus (SFFV) long terminal repeat (LTR)^{28,29} as a transcriptional promoter for the 1.3-kb β -chain transcript from YT35 (ref. 4), which has been shown to be identical to that of Jurkat²⁷. The mammalian selectable marker from the plasmid pSV2-neo (ref. 30) provided positive selection of transfectants (Fig. 3). Protoplast fusion was performed as described previously³¹.

Expression in transfected mutants

Of 15 transfectants, 7 expressed abundant levels of both antigen receptor molecules and T3 antigenic epitopes³². An example of the fluorescence histograms obtained with one such transfected cell, PF-2.8, is compared with those obtained with the wild-type (Jurkat) and the recipient mutant J.RT3–T3.5 (Fig. 4). Note that although the densities of T3 and T-cell antigen receptor determinants in PF-2.8 are less than those observed in the wild-type Jurkat, PF-2.8 expressed abundant levels of both T3 and T-cell antigen receptor determinants compared with the T3/T-cell antigen receptor negative mutant, J.RT3–T3.5. In all seven of the positive transfectants, the relationship between the density of T3 and T-cell antigen receptor epitopes was constant, thus

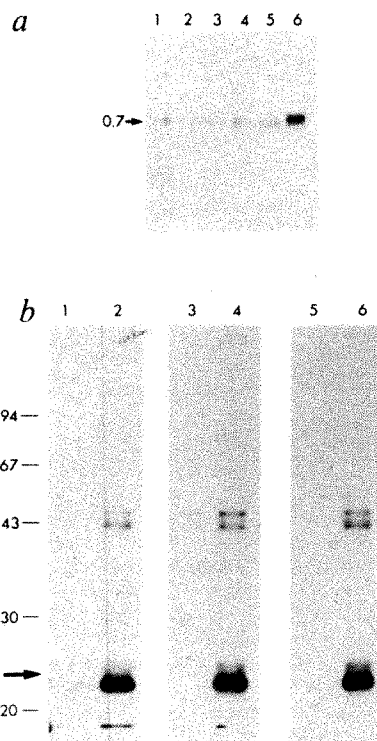


Fig. 1 (Left) *a*, Northern blot analysis of four independently derived T3/T-cell antigen receptor negative mutants of Jurkat hybridized with the T3 δ -chain transcripts. 20 μ g total RNA from the indicated cells were electrophoresed in 1% formaldehyde gels, transferred to nitrocellulose and hybridized with a ^{32} P-labelled cDNA probe specific for the T3 δ -chain^{21,22}. Lane 1, J.EMS-T3.3; lane 2, Jurkat; lane 3, J.RT3-T3.5; lane 4, J.RT-T3.1; lane 5, S.S.; lane 6, HPB-ALL. *b*, SDS-PAGE of biosynthetically labelled T3 antigens in T3 T-cell antigen receptor negative mutants of Jurkat. Lysates of Jurkat (lanes 1 and 2), J.RT-T3.1 (lanes 3 and 4) and J.RT3-T3.5 (lanes 5 and 6) were prepared from cells labelled for 16 h with ^{35} S-methionine and ^{35} S-cysteine. Preformed immune complexes prepared with rabbit anti-mouse IgG and either normal mouse serum (lanes 1, 3, 5) or anti-Leu 4 ascitic fluid (lanes 2, 4, 6) were incubated with each lysate and washed according to previously described methods¹⁸. Isolated complexes were then dissolved in SDS sample buffer containing 2-mercaptoethanol and electrophoresed on a 11% polyacrylamide slab gel. Gels were dried and fluorography was performed using sodium salicylate. Shown in the left margin are the relative mobilities of M_r standards run in parallel lanes. The arrow indicates the 22–23 K doublet representing T3 proteins. Note that the higher M_r bands observed between 40 and 50K are probably contaminants as they are also seen in precipitates isolated with normal mouse immunoglobulin preformed complexes.

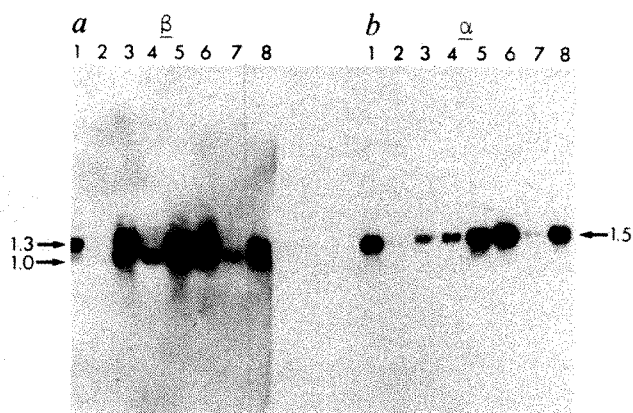


Fig. 2 Northern blot analysis of Jurkat and T3/T-cell antigen receptor negative mutants for β -chain (*a*) or α -chain (*b*) transcripts. 7 μ g poly(A)-enriched RNA were isolated from Jurkat or mutant cells, electrophoresed in a 1% formaldehyde gel, transferred to nitrocellulose paper and hybridized with ^{32} P-labelled nick-translated probe and washed as described elsewhere²². Probes specific for the β -chain (*a*) or α -chain (*b*) of the antigen receptor heterodimer were previously characterized^{4,6}. Lane 1, Jurkat; lane 2, S.S.; lane 3, J.RT-T3.1; lane 4, J.RT-C305.6; lane 5, J.EMS-T3.3; lane 6, J.EMS-C305.1; lane 7, J.RT3-T3.5; lane 8, J.RT3-C305.6. The relative masses (in kb) of the bands obtained are indicated in the margins.

reinforcing their interdependence for their surface expression. The remaining eight transfected clones had either minimal or undetectable densities of determinants recognized by OKT3 or C305 monoclonal antibody.

The clone PF-2.8 that expressed the reconstituted T3/T-cell antigen receptor complex was examined by Southern^{33,34} and Northern^{22,35} blot analyses using a nick-translated³⁵ probe to YT35 (ref. 4) to ensure that the new β -chain mRNA was encoded by the transfected DNA, which can be seen at a new 3.3-kb band in the transfected line PF-2.8 (Fig. 5a, lane 3) that is not present in the mutant J.RT3-T3.5 (lane 2) or the wild-type Jurkat cell (lane 1). Several other new bands can also be detected in

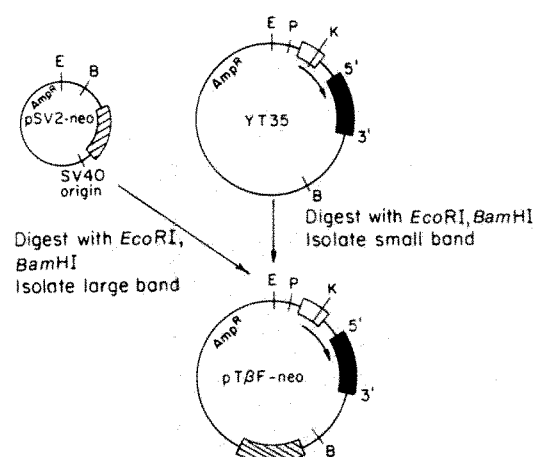


Fig. 3 Construction of plasmid pTBF-neo. The cDNA fragment specific for the β -chain was subcloned into vector pFP502EB511, downstream from the SFFV-LTR, to create plasmid YT35 (ref. 4). pTBF-neo was constructed as illustrated and then transfected into *Escherichia coli* strain DH1. The bacteria were cultured, converted into protoplasts and fused to J.RT3-T3.5 as described in Ochi *et al.*³¹. Cells were plated at a concentration of 10^5 cells per ml in 24-well culture plates and incubated for 48 h. Geneticin was added (2 mg ml^{-1}) to select for transfected clones. □, SFFV-LTR; ■, β -chain cDNA; ▨, neomycin; E, *EcoRI*; B, *BamHI*; P, *PstI*; K, *KpnI*.

PF-2.8, but they probably represent integration events that disrupt the LTR β -chain continuity. These data also show that there are no gross new genomic rearrangements of the β -chain in the mutant J.RT3-T3.5 compared with Jurkat.

Northern blot analysis (Fig. 5b) indicates that two new transcripts homologous with the β -chain are found in PF-2.8 (lane 3). Jurkat, the wild-type cell, contains a 1.3-kb (V-D-J-C rearrangement) and a 1.0-kb (J-C transcript) mRNA (lane 1)²⁷. Lane 2 confirms that the 1.3-kb transcript cannot be detected in J.RT3-T3.5 (ref. 8) and lane 3 shows that the transfected clone expresses new transcripts of 1.9 and 3.4 kb. The increase in size of the β -chain mRNAs presumably results from a

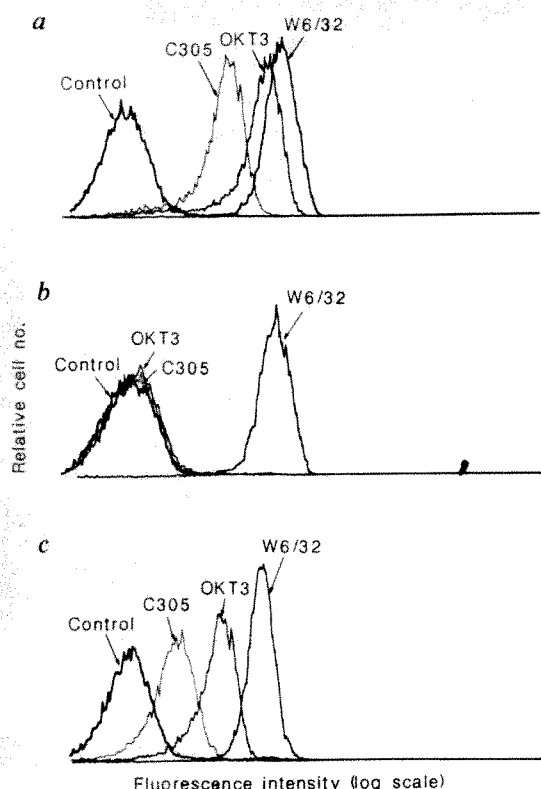


Fig. 4 Immunofluorescence analysis for a T3/T-cell antigen receptor negative mutant of Jurkat transfected with the β -chain cDNA. Initial studies indicated that the SFFV LTR could activate YT35 transcription in the rat cell line Rat-1 (data not shown). Using the technique of protoplast fusion, pTBF-neo was amplified in bacteria and the bacteria fused with the mutant J.RT3-T3.5. This mutant was shown to lack the complete 1.3-kb β -chain transcript and had a decreased level of α -chain mRNA. Transfected cells were selected for geneticin-resistant clones and subsequently expanded in culture. Resistant clones were assayed for surface antigen receptor and T3 with amonoclonal anti-clonotypic antibody, C305 (ref. 16) and monoclonal OKT3 antibody³², respectively, using indirect immunofluorescence as assessed by fluorescence flow cytometry. Jurkat (a); J.RT3-T3.5, a T3 T-cell antigen receptor negative mutant lacking the 1.3-kb β -chain transcript (b); and PF-2.8, a β -chain transfectant of J.RT3-T3.5 (c) were analysed by indirect immunofluorescence using fluoresceinated-Fab'2 goat anti-mouse immunoglobulin on a FACS IV cell sorter. Cells were first stained with a control non-reactive mouse myeloma (MOPC 195) or the indicated antibodies: OKT3 (anti-T3), C305 (anti-receptor) or w6/32 (anti-HLA).

combination of different RNA initiation and termination sites of the newly introduced DNA and makes it unlikely that they represent reactivation of the original β -chain gene.

Initial Northern blot analysis (Fig. 2) indicated that the level of α -chain transcripts was diminished in J.RT3-T3.5. Northern blot analysis of RNA of the transfected line revealed that the level of α -chain transcripts in PF-2.8 (Fig. 5c, lane 3) is comparable in magnitude with that observed in Jurkat (lane 1) and clearly increased from the level observed in the mutant J.RT3-T3.5 (lane 2). These results suggest that transfection of the full-length β -chain into J.RT3-T3.5 induces increased levels of α -chain transcripts.

Reconstituted T3/antigen receptor is active

Previous studies have demonstrated that two stimuli are required for the activation of Jurkat, as measured by interleukin-2 (IL-2) production^{36,37}. One stimulus increases cytoplasmic free calcium $[Ca^{2+}]_i$ and can be provided by the lectin phytohaemagglutinin (PHA), calcium ionophores (A23187 or ionomycin) or monoclonal antibodies reactive with the T3/T-cell antigen receptor complex^{37,38}. The second stimulus can be provided by phorbol

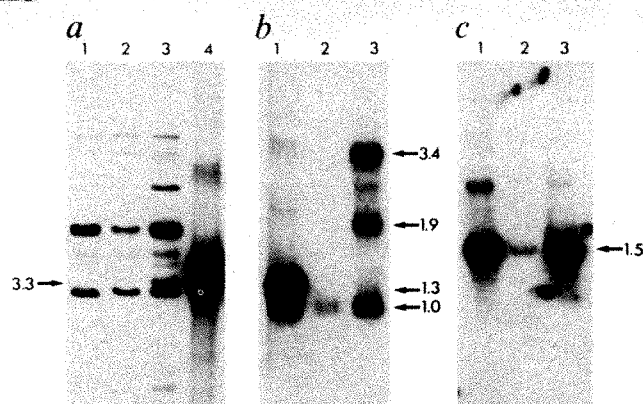


Fig. 5 Molecular analysis of transfectant PF-2.8. **a**, Southern blot analysis. 10 μ g DNA isolated from Jurkat (lane 1), J.RT3-T3.5 (lane 2), PF-2.8 (lane 3) and pTBF-neo (lane 4) were digested with *Eco*RI and *Bam*HI, electrophoresed in an 0.8% agarose gel, transferred to nitrocellulose and hybridized as described³³. The probe was the ³²P-labelled nick-translated ³⁴*Eco*RI/*Bam*HI fragment of pTBF-neo that contains β -chain cDNA. **b**, Northern blot analysis for β -chain transcripts in PF-2.8. 7 μ g poly(A)-enriched RNA was isolated from Jurkat (lane 1), J.RT3-T3.5 (lane 2) and PF-2.8 (lane 3) and was electrophoresed in a 1% formaldehyde gel, transferred to nitrocellulose³⁵ and probed with ³²P-labelled nick-translated β -chain cDNA. **c**, Northern blot analysis for α -chain transcripts in PF-2.8. 7 μ g poly(A)-enriched RNA isolated from Jurkat (lane 1), J.RT3-T3.5 (lane 2) and PF-2.8 (lane 3) were electrophoresed in a 1% formaldehyde gel, transferred to nitrocellulose³⁵, and probed with ³²P-labelled nick translated α -chain cDNA⁶. Numbers in the margins indicate M_r (kb).

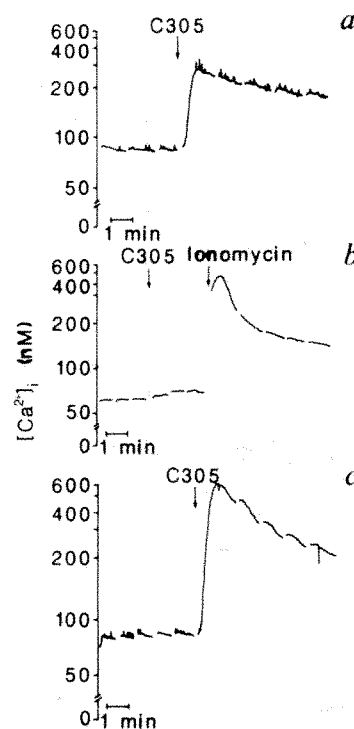


Fig. 6 Changes in $[Ca^{2+}]_i$ in response to anti-receptor (C305) in Jurkat (a); the T3/antigen receptor negative mutant, J.RT3-T3.5 (b); and the β -chain transfectant, PF-2.8 (c). Cells were loaded with the acetoxymethyl ester of quin-2, 20 μ M. Calcium-sensitive quin-2 fluorescence of 10^7 cells per ml was continuously monitored in a Perkins-Elmer 650-40 spectrofluorimeter as previously described³⁷. Interruption in the tracings represent mixture of the cell suspension or addition of C305 (1:300 final dilution of hybridoma culture suspension or addition of C305 (1:300 final dilution of hybridoma culture supernatant) or ionomycin, 1 μ M. $[Ca^{2+}]_i$ was calculated according to the method of Tsien *et al.*³⁹.

myristate acetate (PMA). To investigate whether the reconstituted T-cell antigen receptor on the transfected line PF-2.8 was biologically functional, we measured changes in $[Ca^{2+}]_i$ in response to C305 antibody using quin-2, a calcium-sensitive dye. Whereas C305 induced a prompt and sustained rise in $[Ca^{2+}]_i$ in Jurkat (Fig. 6a), it failed to increase $[Ca^{2+}]_i$ in the T3/T-cell antigen-receptor complex negative mutant, J.RT3-T3.5 (Fig. 6b). However, $[Ca^{2+}]_i$ was increased in J.RT3-T3.5 by the calcium ionophore ionomycin (Fig. 6b). When C305 was added to the transfected line PF-2.8, the increase in $[Ca^{2+}]_i$ was restored (Fig. 6c), suggesting that the reconstituted T3/T-cell antigen receptor complex on PF-2.8 was functional. We have demonstrated previously that mutants of Jurkat that fail to express the T3/T-cell antigen receptor complex fail to produce IL-2 in response to PHA plus PMA^{16,37}. Reconstitution of the T3/T-cell antigen receptor complex restored the PHA responsiveness of PF-2.8 as detected by IL-2 production (data not shown). Thus, the transfection of the β -chain gene resulted in not only reconstitution of the expression of the T3/T-cell antigen receptor structural proteins, but also in a functional receptor.

Discussion

The absence of 1.3-kb β -chain transcripts associated with diminished levels of α -chain transcripts could be explained by a defect in a common regulatory element. In this case, however, the presence of a β -chain transcript regulated by the extraneous promoter should not be able to compensate for a defective regulatory protein at the normal α -chain locus. Alternatively, expression of the β -chain may effect the transcriptional level of the α -chain. Results presented here (Fig. 5) provide support for the latter alternative as transfection of the β -chain cDNA into J.RT3-T3.5 results in an increased appearance of α -chain transcripts. Additionally, because the 1.0-kb β -chain transcripts are made in mutants that fail to contain 1.3-kb β -chain transcripts, it is likely that the genes encoding these transcripts have separate regulatory control.

The reconstitution of the T3/T-cell receptor complex using β -chain cDNA suggests that expression of both α - and β -chain proteins is a requirement for the cell-surface expression of T3 proteins. This is in general agreement with the observation that

the cell-surface appearance of T3 on immature T-leukaemic lines and the thymus is coordinated with the appearance of the T-cell receptor heterodimer².

This intimate association between T3 and the T-cell heterodimer may serve as both a structural and functional molecular complex in T-cell activation. We have demonstrated previously that the T3/T-cell antigen receptor complex initiates activation by mechanisms that lead to increases in cytoplasmic free calcium³⁷⁻³⁹. Triggering the T3/T-cell antigen receptor complex results in polyphosphoinositide metabolism and subsequent release of inositol trisphosphate, which in turn induces a rise in cytoplasmic free $[Ca^{2+}]_i$ by the release of intracellular $[Ca^{2+}]_i$ stores⁴⁰. Thus, the association of T3 and the T-cell antigen receptor may represent both a structural interaction necessary for cell-surface expression and a functional interaction necessary for the mechanisms responsible for increases in cytoplasmic free $[Ca^{2+}]_i$. It is clear that the disulphide-linked heterodimer is involved in an antigen binding function. The role of T3 has not been defined, but we suggest that its function is to initiate activation by causing turnover of polyphosphoinositides and mobilization of intracellular calcium.

Finally, the reconstitution of a biologically active receptor by DNA transfer provide direct evidence that β -chain mRNA encodes a protein that constitutes part of the T-cell receptor, and these data provide a basis for further examination of T3/T-cell antigen receptor as a recognition and signal transduction molecular complex.

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Skyhook gravitational-wave detector

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Gravitational waves in the 10–100-mHz band are inaccessible to Earth-based detectors because of seismic noise. Hitherto, the most sensitive detectors in this frequency band have been the Doppler tracking of interplanetary spacecraft¹ and excitations of seismic motions in the Earth's surface^{2,3}. Here we propose a new and more sensitive type of Earth-orbiting gravitational-wave detector, called a 'skyhook', which would operate in the 10–100 mHz band. The skyhook would consist of two masses, one on each end of a long thin cable with a spring at its centre. As it orbits the Earth, the cable would be stretched radially by the Earth's tidal gravitational field. Gravitational waves would pull the masses apart and push them together in an oscillatory fashion; their motion would be transmitted to the spring by the cable; and a sensor would monitor the spring's resulting motion. (An analogous skyhook, for non-gravitational-wave purposes, was proposed by Colombo⁴ *et al.* 10 years ago and is planned for flight on the space shuttle⁵).

The Doppler tracking¹ of interplanetary spacecraft and seismic motions^{2,3} have r.m.s. noise of $h \sim 3 \times 10^{-13}$ in each case. Hellings has pointed out to us that the evaluation of the h sensitivity of seismic motions in his ref. 1 is incorrect by a factor of $\sim 6\pi$ (Earth radius)/(sonic wavelength). We express the noise level of a gravitational-wave detector in terms of $h = f^{1/2}h(f)$, where $h(f)$ is the square root of the spectral density of the transverse-traceless part of the metric perturbation at frequency f . At the end of this century expensive laser interferometric detectors^{6,7} might fly in space with noise levels $h \sim 10^{-21}$. The skyhook could be a relatively inexpensive and unsophisticated broad-band, Earth-orbiting detector, with sensitivity $h \sim 3 \times 10^{-17}$, intermediate between the present detectors and the envisioned interferometric systems.

By integrating for several weeks, the skyhook could detect the gravitational waves from binary stars with periods of order 1 min and wave amplitudes of the order of 10^{-19} , including, for example, the waves that are predicted to come from Geminga according to the Bisnovatyi-Kogan model⁸ ($0.6M_{\odot}$ white dwarf in a 1-min period orbit around a $5M_{\odot}$ black hole at a distance 100 pc from Earth) or the Nulsen-Fabian⁹ model (two neutron stars in a several-minute-period orbit at 10-pc distance).

Illustrative parameters for the skyhook are: the masses on the cable ends might be $m_0 = 20$ kg. The cable might have a length $L = 25$ km, diameter $d = 0.6$ mm, and cross-sectional area $S = 0.0028$ cm². It might be made of a composite material with thermal expansion coefficient $\alpha = 10^{-7}$ K⁻¹, density $\rho = 3$ g cm⁻³, and Young's modulus $Y = 1 \times 10^{12}$ dyn cm⁻². In this case, the cable's mass would be $m_c = \rho SL = 21$ kg, its speed of longitudinal sound waves would be $v_s = (Y/\rho)^{1/2} = 5.8 \times 10^5$ cm s⁻¹, and the rigidity of its top half (or bottom half) would be $K_c = 2YS/L = 2.3 \times 10^3$ dyn cm⁻¹. The spring at the cable's centre might have a rigidity for each half (top or bottom) of $K_s = 2.3 \times 10^3$ dyn cm⁻¹. Then the effective mass of each half of the system would be $m = m_0 + m_c/4 = 25$ kg, the net spring constant of each half of the system would be $K = K_c K_s / (K_c + K_s) = 1,200$ dyn cm⁻¹, the angular eigen frequency of the system would be $\omega_0 = (K/m)^{1/2} = 0.22$ s⁻¹, and the eigen frequency would be $f_0 = \omega_0/2\pi = 35$ mHz. Internal dissipation in the cable and spring might give the eigen oscillations a quality factor $Q = 10^5$. The tidal force on each half of the system relative to its centre, augmented by the centrifugal force due to the rotation of the skyhook as it orbits the Earth, would be $F_t = m(f/2)\Omega^2 = 9.4 \times 10^4$ dyn where $\Omega = 1.0 \times 10^{-3}$ s⁻¹ is the

skyhook's orbital angular velocity around the Earth. This tidal force would stretch the spring by an amount $x = 2F_t/K_s = 82$ cm.

A gravitational wave with amplitude $h \sim 10^{-17}$ and frequency $f = 2\pi\omega$ in the 10–100-mHz band would produce oscillatory changes of spring length with magnitude $\Delta x \sim m\omega^2 h L / 2K_s \sim 10^{-11}$ cm; this is large enough for a simple sensor to read it out in a time $< 1/\omega \sim 5$ s. Thus, the detector would have a broad-band response.

The length of the cable is limited by the demand that it transmit the gravitational-wave force directly to the spring without excitation of cable sound waves. This requires that the cable length L be equal to about half the sonic wavelength at the highest gravity-wave frequency of interest, $f_{\max}$: that is, $f_{\max} \approx v_s/2L \approx 100$ mHz. The detector would still have some sensitivity at frequencies higher than this, but its response would be debilitated and complicated by the cable's sound waves.

Six noise sources look as though they might produce serious problems for the skyhook: Nyquist noise produced by the fluctuating part of the skyhook's internal dissipation; fluctuations in cable length produced by fluctuations in solar and Earth heating; fluctuation forces due to the gravity gradients of the high harmonics of the Earth; fluctuating electrical and magnetic forces; micrometeoroid impacts; and atmospheric drag.

The spectral density of the Nyquist force acting on each half of the spring is $S_F = 4mkT\omega_0/Q$, where k is Boltzmann's constant and $T \approx 300$ K is the skyhook's temperature. As the spectral density of the gravity-wave force on each half of the spring is $S_F = [m(L/2)\omega^2 h(f)/2]^2$, with $f = \omega/2\pi$, the Nyquist noise expressed in gravitational-wave units is

$$h_{\text{Nyq}} = f^{1/2}h(f) = \left(\frac{32}{\pi} \frac{kT\omega_0}{mL^2\omega^3 Q} \right)^{1/2} = 3 \times 10^{-17} \left(\frac{f}{30 \text{ mHz}} \right)^{-3/2}$$

The cable is continually being heated by radiation from the Sun and Earth (energy flux $F = 1.7 \times 10^6$ erg cm⁻² s⁻¹) and cooled by emission of radiation into space. If the cable is painted white so that its albedo is $A = 0.97$, and if its specific heat is $C = 6 \times 10^6$ erg g⁻¹ K⁻¹, then its thermal response time (e -folding time for temperature to return to equilibrium after a sudden change of irradiating flux) is $t_T = \pi C \rho T d / 32 F (1 - A) = 600$ s. A fractional oscillation $\Delta F/F$ in irradiating flux at angular frequency ω will produce a fractional oscillation $\Delta T/T = (1/4\omega t_T) \Delta F/F = (1/500)(30 \text{ mHz}/f) \Delta F/F$ in the cable's temperature T . This, in turn, will produce a fractional oscillation $\Delta L/L = \alpha \Delta T = 6 \times 10^{-8} (30 \text{ mHz}/f) \Delta F/F$ in the cable's length, which, in turn, will produce an oscillating tidal force on the top or bottom half of the cable $\Delta F_t = 3m\Omega^2 \Delta L/2$. By equating this with the corresponding gravitational-wave force, we see that the noise produced by fractional fluctuations $\Delta F/F$ in irradiating flux at frequency f is $h_{\text{heat}} = 6(\Omega/\omega)^2 (\alpha T/4\omega t_T) \Delta F/F = 1 \times 10^{-11} (30 \text{ mHz}/f)^3 \Delta F/F$. Thus, if the skyhook is to have a sensitivity of $h \sim 3 \times 10^{-17}$ at $f \sim 30$ mHz, the fractional fluctuations in irradiating flux at 30 mHz and in a bandwidth of order 30 mHz must either be below 3×10^{-6} or be monitored by an instrument onboard the skyhook to this sensitivity.

There are no data with the required accuracy on fluctuations in the solar luminosity in the 10–100-mHz band, but at 1 mHz the ACRIM (Active Cavity Radiometer Irradiance Monitor) detector on the SMM (Solar Maximum Mission) spacecraft reveals¹⁰ $\Delta F/F = 30 \times 10^{-6}$, and as one moves upward in frequency by a factor 30, $\Delta F/F$ may well drop off¹¹ by a factor of 10, to 3×10^{-6} . However, for safety one might wish to monitor the Sun's irradiating flux.

Fluctuations in the Earth's surface temperature and albedo with spatial wavenumber $k = 1/40$ km will produce, as the skyhook orbits the Earth with speed 8 km s⁻¹, fluctuations $\Delta F/F$ at frequency 30 mHz. If the skyhook is at an altitude z above the Earth's surface, $\Delta F/F$ will be reduced below the fractional fluctuations of Earth's temperature and albedo by a factor $\sim \exp(-kz)$, which is $< 10^{-6}$ for altitudes > 550 km. Thus, for orbital altitudes above 550 km, fluctuations in the Earth's radiation are probably not a problem.

The radiative heating of the cable will also vary due to fluctuations in the direction of the cable. However, it seems unlikely that anything will produce fluctuations on the relevant timescale large enough to be a problem.

Of course, whenever the skyhook moves into and out of the Earth's shadow its temperature will change by $\Delta T/T \sim 1/3$; and afterward the cable will require a time of order $t_r \ln 10^8 \sim 3.1$ h to settle back down to a length sufficiently steady for gravity-wave observations. As the orbital period is only ~ 1.7 h, gravity-wave observations will be possible only from a high-inclination orbit where there are long stretches of non-shadow time.

Heating and cooling due to passage through the shadow will probably enhance sudden strain releases (creep) in the cable, and the presence of such creep may necessitate doing experiments with two or more skyhooks simultaneously and cross-correlating their data. Fortunately, each shadow passage will probably cause some annealing of the cable; and, as a result, creep will probably become less serious as the cable ages.

If the skyhook is flown in too low an orbit, noise from the Earth's gravity gradients will be a problem. Fluctuations, $\Delta\sigma$, in the Earth's surface density of mass with wavenumber $k = \omega/v_{\text{orb}} = (1/40 \text{ km})(f/30 \text{ mHz})$ will produce a tidal force on the top (or bottom) half of the skyhook $\Delta F_t = \pi m G \Delta\sigma (kL) \exp(-kz)$. Equating this to the gravity-wave force $m\omega^2 hL/4$, we obtain for the gravity-gradient noise in gravitational-wave units, $h_{\text{gg}} = (4\pi G \Delta\sigma / \omega v_{\text{orb}}) \exp(-kz)$. For $\Delta\sigma = 3 \times 10^5 \text{ g cm}^{-2}$ and $f = 30 \text{ mHz}$, a skyhook altitude of $z > 1,000 \text{ km}$ will keep $h_{\text{gg}} < 10^{-17}$. The skyhook could function as a good gravity-wave detector at lower altitudes only after the Earth's gravity gradients on scales $k \sim 1/40 \text{ km}$ have been adequately mapped.

Of course, the skyhook itself could be used to map the Earth's gravity gradients, but only one of their six components, the radial-radial component. The skyhook's sensitivity to this component would be $\Delta\Phi_{rr} = \omega^2 h/2 \sim 5 \times 10^{-19} \text{ s}^{-2}$ in a bandwidth $\Delta f \sim f \sim 30 \text{ mHz}$, which corresponds to $\sim 3 \times 10^{-18} \text{ s}^{-2} \text{ Hz}^{-1/2}$. This is far more accurate than the $10^{-13} \text{ s}^{-2} \text{ Hz}^{-1/2}$ sensitivity of the best gravity gradiometer being developed for flight in space¹², but that gradiometer would measure all six components of the Earth's tidal field, not just the radial-radial component.

The earth's magnetosphere exhibits $f = 10$ – 100 -mHz standing-wave Alfvénic excitations, called Pc2,3,4 and Pil,2 micropulsations¹³, which will interact with the skyhook to produce debilitating noise. On magnetic field lines which cross the geomagnetic equator at ≥ 3 Earth radii ($L \geq 3$ field lines) these micropulsations are present about 50% of the time and have electrical and magnetic field amplitudes $\Delta B \sim (0.1\text{--}100) \text{ nT} = (10^{-6}\text{--}10^{-3}) \text{ G}$; $\Delta E = [(\text{Alfvén velocity})/c] \Delta B \sim (0.1\text{--}100) \text{ mV m}^{-1}$ (refs 13, 14). On field lines with $L \leq 3$, the $f = 10$ – 100 mHz micropulsations may be less frequent and weaker,^{14,18} and presumably the predominant quiet-time fields have $\Delta B \leq 0.03 \text{ nT}$, $\Delta E \leq 0.03 \text{ mV m}^{-1}$.

If the skyhook's cable is a good conductor (resistivity $\mathcal{R} = 10^{-5} \text{ ohm cm}$; resistance $R_c = \mathcal{R}L/S = 9,000 \text{ ohm}$), then the electromotive force $\mathcal{E} = (v/c)BL = 6,000 \text{ V}$ caused by its $v = 8 \text{ km s}^{-1}$ motion through the Earth's $B = 0.3 \text{ G}$ field, will drive current to flow from the magnetosphere into one end region of the cable, along the cable, and out the other end region. Although the magnetospheric impedance for this flow is not well understood (refs 5, 15 and P. M. Banks, personal communication), it will surely be high enough to force the cable to develop a vertical gradient of charge density such that $\Delta q' = (\text{mean charge per unit length on upper half}) - (\text{mean charge per unit length on lower half}) \gg 10^{-3} \text{ ESU cm}^{-1}$. Such a charge difference, interacting with quiet-time electric-field fluctuations $\Delta E_z \sim 0.03 \text{ mV m}^{-1}$, would produce noise at the level $h_{\text{EM}} \gg 10^{-15}$. Thus, the cable must not be a good electrical conductor.

A nonconducting cable, like any satellite, will charge up to $V_0 \sim (a \text{ few } kT_e/e) \sim 1 \text{ V}$ in the plasma rest frame because the electron thermal velocity $(3kT_e/m_e)^{1/2}$ exceeds that of the ions and because of photoelectron emission from the cable's surface^{15,16}. The resulting charge per unit length on the cable will

have magnitude $q' \sim 4 \times 10^{-4} \text{ ESU cm}^{-1}$. This charge (essentially equal on the two halves of the cable) will interact with the vertical gradient (d/dz) of the vertical electric-field fluctuations ΔE_z to produce a stretching of the spring. The stretching force on the top (or bottom) half of the spring will be $F_{\text{EM}} \approx (d\Delta E_z/dz)(L^2/8)q'$. Equating this to a gravitational-wave force $\Delta F = m\omega^2 Lh/4$, we find for the electromagnetic noise in gravitational-wave units

$$h_{\text{EM}} \approx 3 \times 10^{-17} \left[\frac{L(d\Delta E_z/dz)}{0.004 \text{ mV m}^{-1}} \right] \left[\frac{30 \text{ mHz}}{f} \right]^2 \left[\frac{V_0}{1 \text{ V}} \right]$$

If the length scales for the vertical gradients of ΔE_z at quiet times are $\geq 10L = 250 \text{ km}$ and if $\Delta E_z \leq 0.03 \text{ mV m}^{-1}$, then h_{EM} will be $\leq 3 \times 10^{-17}$. However, during some micropulsations h_{EM} will be $\gg 3 \times 10^{-17}$. Thus, it will be necessary to carry on board the skyhook one or more electrical field probes to monitor ΔE_z ; and the skyhook orbit should be chosen to spend as much time as possible in the quiet region $L < 3$, that is $z < 6,400 \text{ km} \times (3 \cos^2 \theta_m - 1)$, where z is the orbital altitude and θ_m is the magnetic latitude.

To recapitulate, the orbital altitude must be $z \geq 1,000 \text{ km}$ to avoid gravity-gradient forces, and $z \leq 6,000 \text{ km}$ to maximize the time spent in quiet E -field regions. The inclination of the orbit to the Earth's Equator, i , must be large enough to permit a reasonable fraction of all orbits to be in full sunlight, $i \geq 90^\circ - \sec^{-1}(1 + z/R_\oplus) \approx [60^\circ \text{ if } z = 1,000 \text{ km}; 30^\circ \text{ if } z = 6,000 \text{ km}]$, but small enough (preferably $i \leq 65^\circ$) to minimize the time spent on noisy field lines with $L \geq 3$.

Approximately once per day the skyhook will encounter a swarm of micrometeoroids with individual masses $m \sim 10^{-13} \text{ g}$.¹⁷ The ~ 10 impacts per second during a ~ 10 min encounter will mimic gravity waves with $h \sim 3 \times 10^{-16}$ at $f \sim 30 \text{ mHz}$. Such impacts may be identifiable by the time development of the data; and they can be anticorrelated by cross correlating two skyhooks.

Air molecules striking the cable will transfer to it an average fraction $\mu = (\text{accommodation coefficient})$ of their longitudinal momentum. Correspondingly, vertical 'winds' in the residual atmosphere, with wind velocity fluctuations Δv_w and with scale height H for vertical momentum density, will produce a fluctuating longitudinal drag force which mimics gravitational waves with $h \sim [\mu(\Delta v_w/v_{\text{orb}})(L/8H)F_{\text{drag}}][m\omega^2 L/4]^{-1}$. Here F_{drag} is the total neutral-atmosphere-induced drag on the skyhook. This fluctuating drag may require the skyhook to fly at altitudes $z \geq 2,000 \text{ km}$, where $F_{\text{drag}} \leq 0.02 \text{ dyn}$, $H \geq 1,000 \text{ km}$, and

$$h \sim 3 \times 10^{-17} \left(\frac{\mu}{0.1} \right) \left(\frac{1,000 \text{ km}}{H} \right) \left(\frac{F_{\text{drag}}}{0.02 \text{ dyn}} \right) \left(\frac{\Delta v_w}{25 \text{ m s}^{-1}} \right) \times \left(\frac{30 \text{ mHz}}{f} \right)^2$$

and even then correlation drag fluctuations may require two-skyhook correlations for their removal. Charged-particle drag, which is much more difficult to analyze and is intimately connected to electromagnetic noise, might also be a problem.

Noise due to forces on the cable (drag, micrometeoroid, and electromagnetic) might be strongly reduced by using a more complicated skyhook design (suggestion by R.W.P. Drever): use a single cable (no central spring) with one spring at each end between cable and end mass; make the cable much stiffer than the two springs, $k_c \gg k_s$, so it acts as a rigid rod connecting them; and add the displacements of the springs.

The skyhook is, in summary, an attractive, simple instrument with gravity-wave sensitivity in an interesting range where sources might exist but are not guaranteed to exist.

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The geomagnetic mass spectrometer—mass and energy dispersions of ionospheric ion flows into the magnetosphere

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NASA's Dynamics Explorer (DE) mission was designed to study the coupling between the Earth's magnetosphere, ionosphere and neutral thermosphere¹. One area of major interest is the outflow of ionospheric plasma into the magnetosphere, the scale and significance of which is only now becoming apparent with the advent of mass-resolving, low-energy ion detectors. Here we compare observations of ion flows in the polar magnetosphere, made by the retarding ion mass spectrometer (RIMS)² on DE1, with those made simultaneously in the topside ionosphere by the ion drift meter (IDM)³ on the lower-altitude DE2 spacecraft. The results show the dayside auroral ionosphere to be a significant and highly persistent source of plasma for the magnetosphere. The upwelling ionospheric ions are spatially dispersed, according to both their energy and mass, by the combined actions of the geomagnetic field and the dawn-to-dusk convection electric field, in an effect analogous to the operation of an ion mass spectrometer.

The two DE satellites were launched into co-planar polar orbits on 3 August 1981; DE1 being in a highly elliptical orbit which carried it out to a geocentric distance, r , of 4.5 Earth radii (R_E), while DE2 remained at altitudes below about 1,000 km. During the first 18 months of the mission, the DE1 apogee precessed from the north to the south pole.

The RIMS instrument on DE1 comprises three detector heads, each an ion mass spectrometer with a potential analyser input, and each capable of observing ions of two mass-per-charge ratios simultaneously. Ion species of mass up to 32 AMU can be resolved and for each species an integral energy spectrum over the range 0–50 eV (relative to spacecraft potential) is obtained. The RIMS radial head sweeps through a full range of pitch angles twice during each satellite spin period of 6 s: Fig. 1 shows data from this radial head for a pass from north to the south polar regions on 3 April 1982. By this date, DE1 perigee was close to the geomagnetic equator (time C) and no data were obtained between times B and D, when the ambient

plasma density was too great for RIMS to operate. Figure 1 shows contours of O^+ and H^+ ion counts per accumulation period (12 ms) as a function of time and satellite spin angle. Count rates are approximately proportional to integral ion flux, one count per sample being roughly equivalent to 3.1×10^4 and $4.4 \times 10^4 \text{ cm}^{-2} \text{ s}^{-1} \text{ sr}^{-1}$ for H^+ and O^+ respectively; during most of this pass they were largest when the spin angle was close to zero, that is, when the radial head was looking along the orbit path or RAM direction. This indicates that the plasma was cold and isotropic and that ion flow velocities were much smaller than the spacecraft speed ($\sim 7 \text{ km s}^{-1}$ at $r = 2R_E$ and 10 km s^{-1} at $r = 1.1R_E$)⁴. Exceptions to this were the behaviour of H^+ ions in the northern polar cap (before 1157 UT, the time A), for which the peak count rate was shifted from the RAM direction towards the upward field-aligned direction (the upper of the two dashed lines), revealing upward field-aligned flow, as predicted for the light-ion polar wind^{5,6}; in addition the peak O^+ ion count rates near both magnetic poles (before about 1150 UT and after about 1240 UT) are shifted towards the downward field-aligned direction, indicating the ions are moving downward under the influence of gravity⁷. However, the largest flow features were seen at times A and E when DE1 passed through the morning sector auroral ovals in each hemisphere and strong upward flows were observed of both O^+ and H^+ ions.

These upflows have been termed 'upwelling ion events'⁴ as all ion species show the effects of equal ion heating below the satellite (to parallel and perpendicular temperatures of ~ 2 and 10 eV, respectively) and carry an upward heat flux. Ion species observed streaming upwards in these events include H^+ , He^+ , O^+ , N^+ and O_2^+ (ref. 7), and there is evidence that molecular ions N_2^+ , O_2^+ and NO^+ , seen by RIMS in the polar magnetosphere, may also originate from the events. The number fluxes of upwelling O^+ are very large, requiring of the order of 10^9 ions $\text{cm}^{-2} \text{ s}^{-1}$ out of the topside ionosphere, which is an order of magnitude larger than the cold polar wind flux summed over all ion species^{5,6}: the total O^+ outflow from both hemispheres has been estimated to average over 10^{25} s^{-1} which compares with an estimated polar wind outflow of $5 \times 10^{25} \text{ s}^{-1}$. A statistical survey of 2 years' RIMS data has shown that these events are a highly persistent phenomenon and are only found in a narrow region near the morning and noon polar cap boundaries⁴.

The interaction of the solar wind, and the interplanetary magnetic field (IMF) embedded within it, with the Earth's magnetosphere generates large-scale electric fields which give 'convection' of plasma at high latitudes. If the IMF has a southward component, this flow is anti-sunward over the polar cap.

Solar wind ions injected into the magnetosphere in the dayside auroral zone have been observed to be dispersed in mass, energy and pitch angle by this poleward motion⁸: lower velocity ions are found further poleward because their time of flight is greater and hence they are convected over greater distances. This effect should also be observed for the upwelling ionospheric ions described above, particularly at great altitudes where the dispersion should be larger.

Early in the DE mission, RIMS afforded an excellent opportunity to see dispersion of upflowing ionospheric ions, with DE1 apogee being over the northern polar cap. Figure 2 shows data from 22 October 1981 when DE1 and DE2 were in close magnetic conjunction over the northern dayside auroral region. The level of geomagnetic activity was moderately high ($K_p = 5$), for which the occurrence probability of upwelling ions is very close to unity⁴: the IMF was (and had previously been) predominantly southward, giving anti-sunward convection, as was observed by the IDM on DE2.

Figure 2a shows the retarding potentials required to reduce the count rates from the RIMS radial head to below two per sample, for upward field-aligned flows of H^+ , He^+ and O^+ ions near 22,000 km in altitude; these are estimates of the peak energy for each ion species. Figure 2c shows the field-aligned flow velocity and flux, as seen by the IDM during the DE2 pass at an altitude near 900 km. The arrows relate the observations to

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Fig. 1 Spin angle/time plots of count rate contours for O^+ and H^+ ions, observed during a pole-to-pole pass of DE1 on 3 April 1982 by the RIMS radial head. Zero spin angle corresponds to the satellite orbit direction and the upward field-aligned direction is shown by the dashed line in the Southern Hemisphere ($MLAT < 0$) and by the dashed-and-dotted line in the Northern Hemisphere. For each universal time (UT), the geocentric distance (r), magnetic local time (MLT) geomagnetic latitude (MLAT) and invariant latitude (Λ) of DE1 are shown.

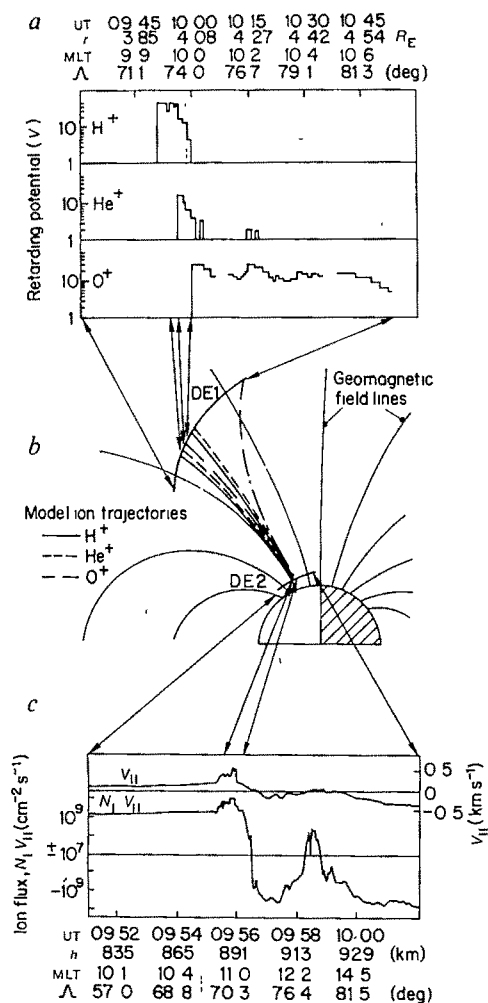
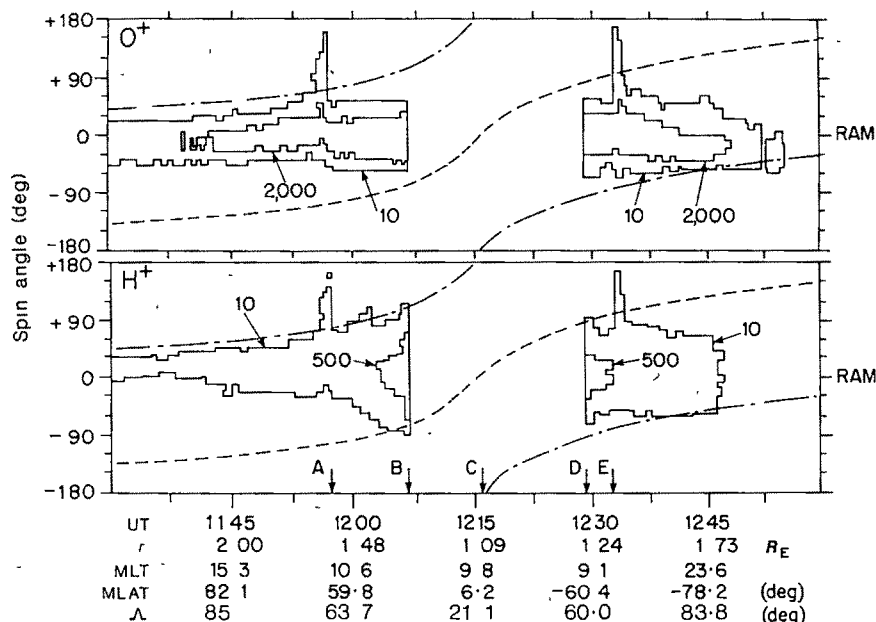


Fig. 2 *a*, Retarding potential required to reduce ion count rates observed by RIMS radial head below two per accumulation period (12 ms) for a high-altitude, polar pass of DE1 on 22 October 1981. *b*, Results for field-aligned flows of H^+ , He^+ and O^+ ions. *c*, Field-aligned ion velocity ($V_{||}$) and the flux ($N_{||}V_{||}$) observed simultaneously by the IDM on DE2. Positive values are upward. Orbital tracks and model ion trajectories for this period are shown projected onto the noon-midnight meridian in *b*. The ion trajectories shown are computed for the highest and lowest energy ions of each species as observed by RIMS. Λ is the invariant latitude of the spacecraft and h is the altitude.

the locations of the satellites, projected onto the noon-midnight meridional plane in Fig. 2*b*. As DE1 moved polewards, RIMS observed first H^+ , then He^+ and finally O^+ ions at any one energy and, in addition, the peak ion energy decreased for each ion species. Hence these observations qualitatively show the predicted mass and energy dispersions. That these data are quantitatively consistent with the 'geomagnetic mass spectrometer' concept is demonstrated by the model ion trajectories also shown in Fig. 2*b*. These have been computed from the ion energies observed by RIMS using a two-dimensional kinetic model⁹ with a dawn-to-dusk electric field consistent with the IDM observations of field-perpendicular ion flow. It is assumed that the ion motion is collision free and that the electrostatic potential distribution along the geomagnetic field lines of the polar cap is as predicted for the cold polar wind⁵, as has recently been inferred from other RIMS data⁷. It can be seen that all ion trajectories converge to a narrow ionospheric source region where an exceptionally large ion upflow signature was observed by the IDM (total ion flux exceeding $10^9 \text{ cm}^{-2} \text{ s}^{-1}$). Figure 2 shows that O^+ ions were found throughout most of the dayside polar cap where RIMS data were available for this pass of DE1; other passes at high Kp (≥ 5) also reveal O^+ ions throughout the polar caps⁷.

These observations show that the narrow ionospheric source region acts as the collimation slit of the geomagnetic mass spectrometer which can disperse ions, particularly cold and heavy ions, throughout the polar cap; such O^+ ions have already been observed by both ion detectors on DE1^{10,11}. Lastly, note that these ions are present in addition to (and are warmer than) the light ions of the classical cold polar wind, which can only be observed by RIMS at these altitudes with the application of an input aperture bias potential, which overcomes the repulsive effect of the positive spacecraft potential¹².

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Raman intensity measurements for determining conformer populations as a function of pressure

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Raman spectroscopy of fluids under pressure has been increasingly studied^{1,2} and applied^{3,4} over the past few years. Essential to the extension of these investigations is the reliable interpretation of the pressure-induced spectral changes of model systems in terms of variations in conformation and molecular dynamics. Conformational investigations have been made on systems that were suspected of undergoing conformational change at high pressures, for example, *n*-alkanes⁵⁻⁹, haloalkanes¹⁰⁻¹³ and substituted cyclohexanes¹⁴⁻¹⁹. Raman investigations reported here reveal significant pressure-induced spectral changes in both conformationally sensitive and insensitive systems. We suggest that these arise from a breakdown in the tenet of the pressure invariance of a direct proportionality between Raman scattering intensity and concentration. This is most apparent from the differential behaviour of symmetrical and antisymmetrical vibrations. We propose a re-evaluation of some previous interpretations of the pressure-induced spectral changes.

The vibrations of polyatomic molecules are usually discussed in terms of concerted combined motions of atoms, termed normal modes, where each normal mode forms the basis for an irreducible representation of the point symmetry group of that molecule. In general, a molecule with *n* atoms will possess $3n - 6$ normal modes or $3n - 5$ for linear molecules. Corresponding to each normal mode, a so-called normal coordinate *Q* is introduced that allows the various physical features of the molecule (such as potential energy, polarizability and dipole moment) to be represented as a function of a single length dimension.

For conformationally variant molecules, the essential assumption required for effective interpretation of the spectral changes induced by pressure is that the concentration *C* of a given conformer *r* should be directly proportional to a normalized intensity I_{rk}^{obs} for the *k*th normal mode of that conformer.

$$C_r = a_{rk} \cdot I_{rk}^{obs}$$

where a_{rk} is the proportionality constant.

For Raman spectroscopy, the intensity of an observed vibrational band is, to a first approximation, directly proportional to the derivative of the molecular polarizability (itself a second rank tensor) with respect to the normal coordinate, at the equilibrium position. Thus, for the above assumption to hold, the polarizability change corresponding to a given vibrational mode must itself be independent of pressure.

$$(\delta\alpha/\delta Q_k)_0 = \text{constant}$$

where α is the polarizability tensor, Q_k is the normal coordinate for the *k*th vibrational mode, and the subscript 0 implies the differential polarizability change at the equilibrium position.

In most investigations reported, there has been no attempt to verify the above assumption. Indeed, in the only investigation that does attempt this⁹, it is not clear that the chosen reference bands possess a sufficiently dissimilar character and symmetry to be broadly representative.

We have carried out some detailed high-pressure Raman investigations of a wide range of fluids (see Table 1). We included a comprehensive set of disubstituted cyclohexanes to allow a systematic assignment of some of the spectral features to particular conformational species. As a test of any features assigned in conformationally variant systems, for example, fluids F3, F4 and F7, comparisons were to be made with related conformationally invariant systems, for example, fluids F2, F5 and F6.

We have performed extensive tests to ensure that we have eliminated all contributions deriving from stress-induced birefringence and other optical artefacts. We used two separate Raman microscope systems, scanning and multichannel, and two different diamond anvil cells in addition to a traditional hydrostatic equilibrium cell¹⁹. In all cases, the essential observations were the same.

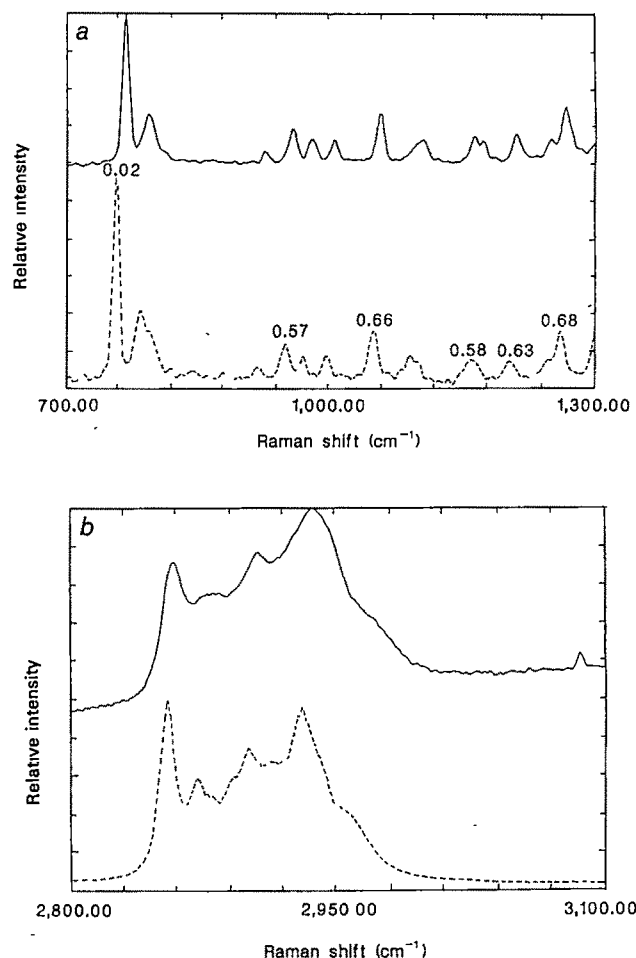


Fig. 1 Raman spectra of *cis*-1,4-dimethylcyclohexane: *a*, optical skeletal region; *b*, C-H, region 180° scattering, 514.5-nm excitation: ---, 0.001 kbar; —, 5.5 kbar. The numbers superimposed on the *a* spectrum are the depolarization ratios corresponding to the major peaks.

Table 1 Fluid number and composition

Fluid no.	Composition
F1	Methyl cyclohexane
F2	<i>cis</i> -1,2-dimethylcyclohexane
F3	<i>trans</i> -1,2-dimethylcyclohexane
F4	<i>cis</i> -1,3-dimethylcyclohexane
F5	<i>trans</i> -1,3-dimethylcyclohexane
F6	<i>cis</i> -1,4-dimethylcyclohexane
F7	<i>trans</i> -1,4-dimethylcyclohexane
F8	Toluene
F9	Toluene d-8
F10	Cyclohexylmethanol
F11	Methyl cyclohexane carboxylate
F12	Methyl cyclopentane carboxylate
F13	Ethyl hexanoate
F14	2,4-dicyclohexyl, 2-methylpentane

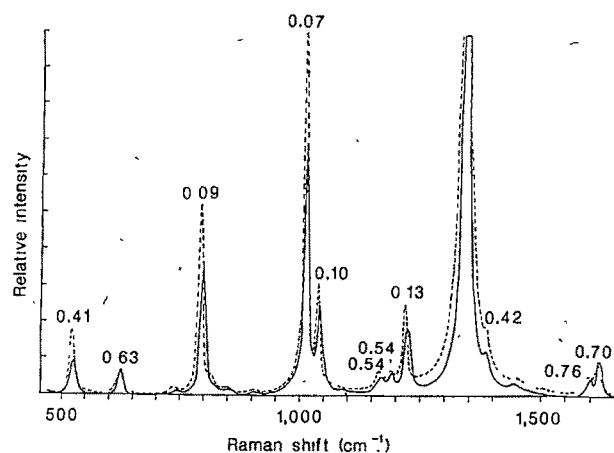


Fig. 2 Raman spectra of toluene, skeletal region, 180° scattering, 514.5-nm excitation: ----, 0.001 kbar; —, 12.8 kbar.

Examples typifying our observations come from two conformationally invariant systems, notably *cis*-1,4-dimethylcyclohexane (F6) and toluene (F8). Figures 1a and 1b depict the ambient and high-pressure (5.5 kbar) Raman spectra of F6 in the optical skeletal (OS) and C-H stretching regions. The numbers superimposed on the OS spectra are the depolarization ratios corresponding to the major features. Figure 1a shows the decrease in integrated intensity of the 765 cm⁻¹ ring breathing band ($\rho = 0.02$) relative to the remaining bands with depolarization ratios in the range 0.57–0.68. In the C-H stretching region (Fig. 1b), the effect is more marked with the 2,855 cm⁻¹ symmetrical CH₂-stretching mode dramatically losing intensity relative to the antisymmetrical 2,931 cm⁻¹ band.

Figure 2 shows toluene, ambient and high-pressure spectra with the corresponding assigned depolarization ratios. Several features are apparent, most notably the loss in relative integrated intensity of the 1,004 cm⁻¹ (A_1 , $\rho = 0.07$), 1,030 cm⁻¹ (A_1 , $\rho = 0.10$) and 1,208 cm⁻¹ (A_1 , $\rho = 0.13$) bands compared with the 1,155 cm⁻¹ (B_1 , $\rho = 0.54$), 1,178 cm⁻¹ (A_1 , $\rho = 0.54$) doublet, and also the 521 cm⁻¹ (A_1 , $\rho = 0.41$) compared with the 623 cm⁻¹ (B_1 , $\rho = 0.73$) bands.

Finally, in a more complex molecule, 2,4-dicyclohexyl-2-methylpentane, originally suspected of conformational change at high pressures³, Fig. 3 illustrates the loss of intensity of the CH₂-symmetrical mode (2,850 cm⁻¹) compared with the CH₂-antisymmetrical region (2,930 cm⁻¹) with its virtual disappearance at pressures approaching 50 kbar.

These examples reveal the relationship between pressure-induced intensity reduction and the symmetry, and hence depolarization ratio, of a given Raman band. Therefore, in many instances one observes that ($I_{\text{sym}}/I_{\text{asym}}$) decreases as pressure increases. However, this effect is most marked for the C-H stretching region. Further observations may be noted for Raman bands of similar depolarization ratios but different vibrational assignments. For example, the C=C bands of a range of unsaturated ester fluids (not listed) were significantly suppressed relative to the C=O stretching bands, despite possessing similar depolarization ratios (~ 0.25).

A qualitative explanation for these observations is available in that the loss of intensity of a given vibrational band as a function of pressure is directly related to the volume change associated with that vibration. Thus, symmetrical modes, in general, and C-H stretching modes, in particular, with large associated volume changes are expected to be suppressed at elevated pressures.

Considerable attention has been given to the effect of pressure on vibrational frequencies of molecular systems. In general, the positive frequency shifts observed with increasing pressure are explained in terms of decreases in equilibrium bond lengths and consequent increases in bond force constants, related to the anharmonicity of the vibrational potential well^{20,21}. In contrast,

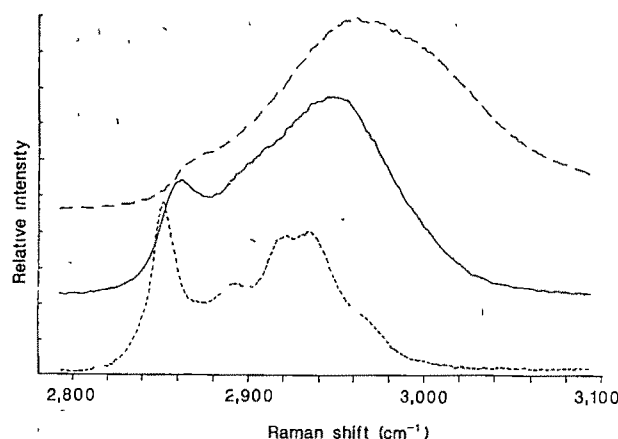


Fig. 3 Raman spectra of 2,4-dicyclohexyl-2-methylpentane, C-H region 180° scattering, 514.5-nm excitation. ----, 0.001 kbar; —, 17.3 kbar; - · - · -, 40.2 kbar.

very little theoretical attention has been focused on the effect of pressure on vibrational band intensities, particularly in fluids, despite the fact that it is well known that in studies of vibrations in molecular crystals symmetrical modes are suppressed relative to the liquid state and are further suppressed by pressure^{22–24}. It is apparent that as pressure induces significant changes in molecular bond lengths such as to cause appreciable force constant changes and consequent vibrational frequency shifts, the shape and slope of the polarizability/normal coordinate curves themselves must become altered. The change in $(\delta\alpha/\delta Q_k)_0$ thus induced must cause intensity changes. This will, therefore, negate the original assumption of a pressure invariance of the concentration/intensity relationship.

These observations raise additional explanations for reported pressure-induced spectral changes. As an example of one of the most thorough conformational investigations reported, one may consider the study by Wong *et al.*⁹ of the longitudinal acoustic mode (LAM) region of the Raman spectrum of *n*-hexane. Their most striking observation was of a considerable reduction in intensity of the band caused by the all-*trans* (*ttt*) conformer relative to bands resulting from the other conformers containing one or two gauche arrangements. However, the key (*ttt*) band used in the population measurement corresponds to a highly symmetrical concertina-like vibration of the molecule with a large associated molecular volume change. In contrast, the remaining bands of this LAM region, whose relative normalized intensities did not vary so significantly, correspond to vibrations of significantly lower symmetry and smaller associated volume change. Our observations on conformationally invariant systems suggest that their tenet of direct proportionality between intrinsic intensity and concentration is not upheld, particularly considering vibrations of such dissimilar symmetry and associated volume change.

Some recent infrared investigations²⁵, again including studies of some conformationally invariant systems, have revealed a similar character of spectral changes as a function of pressure to those observed by Raman spectroscopy. Assignment of the infrared bands to symmetrical and antisymmetrical vibrations is much more difficult, but notably in the C-H region where the intensity changes are most marked, the general behaviour is identical to the Raman data presented here. Thus, our main assertions have wider application to all vibrational spectroscopic studies of fluids under pressure.

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¹⁰Be in ice at Vostok Antarctica during the last climatic cycle

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We have previously reported accelerator mass spectrometer measurements of the cosmogenic isotope ¹⁰Be (half life 1.5 Myr) in an 906-m ice core recovered at Dome C, Antarctica¹. In addition to an enhanced concentration during a period of reduced solar activity (Maunder minimum, AD 1645-1715)², we found substantially increased concentrations during that part of the core corresponding to the last ice age. Both effects have been confirmed and extended in Greenland ice cores^{3,4}. The recovery of a 2,083-m ice core at Vostok, Antarctica⁵, together with an extended oxygen isotope chronology reported elsewhere in this issue⁶, permits an extension of ¹⁰Be studies over the whole of the last climatic cycle (~125 kyr). We report here such measurements, which show an excellent correlation with the oxygen isotope and thus, presumably, climate record over the whole of this period. The results imply that precipitation rates in the Antarctic during the last interglacial were similar to those of the Holocene, but were roughly halved during the last glaciation.

A total of 22 samples, weighing from 0.7 to 2.0 kg, were available for the ¹⁰Be study. After the addition of 0.5 mg of ⁹Be carrier, the samples were treated as described previously¹. The ¹⁰Be measurements were made on a General Ionex Tandemron accelerator mass spectrometer system, using the procedure described elsewhere⁷. The uncertainties have been calculated using a 7% instrumental uncertainty (conservatively estimated on the basis of the reproducibility of a standard and several of the ice samples), together with 1 s.d. statistics on the number of ¹⁰Be events counted. Because we consider that the chronology of the core is still uncertain, no corrections for decay of ¹⁰Be have been made. However, using the present best estimate of the age of the ice (see below), such a correction would be <7% for the deepest sample measured. The results are shown in Fig. 1, together with the oxygen isotope profile for the same core⁶ (note that the ¹⁰Be concentration scale has been inverted to facilitate comparison with the ¹⁸O record). The correlation between the two curves is remarkable. Not only does the ¹⁰Be concentration return to Holocene levels from 1,650 to 1,875 m (believed to represent the last interglacial), but the coldest periods during the glacial, as indicated by the oxygen isotope profile, are also reflected by maxima in ¹⁰Be concentrations.

We have previously discussed three possible explanations for ¹⁰Be concentration variations in ice¹. One involves production

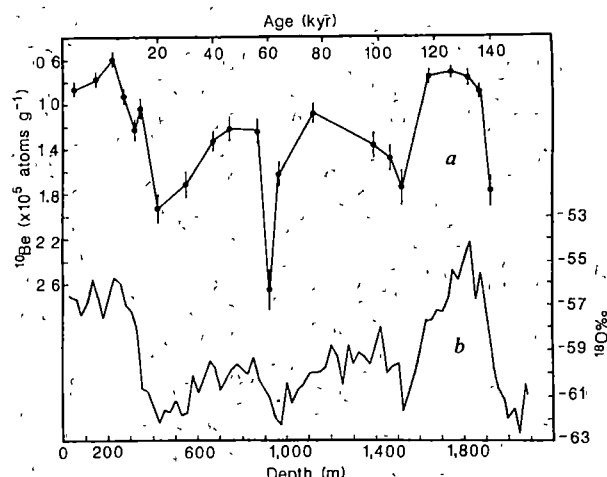


Fig. 1 Concentrations of ¹⁰Be (a) and $\delta^{18}\text{O}$ (b) (from ref. 6) as a function of depth in ice at Vostok. Note inverted scale for ¹⁰Be concentration to facilitate comparison of the two curves. Time scale is that adopted in ref. 6.

variations, possibly induced by solar modulation effects; the second would result from changes in atmospheric circulation; and the third links the concentration variations to changes in precipitation rates. Although we cannot yet exclude effects caused by the first two factors, the present results reinforce our belief that the third mechanism is the most probable explanation for the observed long-term correlation of ¹⁰Be with climate. Indeed, if one can neglect, or correct for, ¹⁰Be flux variations, ¹⁰Be concentrations may be used to estimate past precipitation rates^{8,9}. Such a calculation is shown in Fig. 2, using the data of Fig. 1 and assuming that the deposition flux of ¹⁰Be at Vostok has, on average, been constant over the last climatic cycle.

This interpretation of the ¹⁰Be results implies that the precipitation rate at Vostok during the last ice age was ~50% of that during the present and previous interglacial. This is consistent with results based on conductivity measurements of annual layers in the Byrd core, which indicate about a factor of 2.5 decrease going from the early Holocene to the late glacial period (C. Hammer, personal communication). It is also in partial agreement with the results of Wilson and Hendy who deduced a decrease in annual layer thickness during the late glacial period at Vostok based on sodium variations¹⁰. However those authors also found a precipitation rate larger than the Holocene value at ~900 m which is not indicated by the ¹⁰Be results. Thompson *et al.*¹¹, using annual layers derived from particle concentrations, found an approximately constant precipitation rate during the late glacial period and Holocene at Dome C. As mentioned earlier, our own ¹⁰Be results at Dome C¹ suggest about the same reduction in precipitation during the glacial period as at Vostok.

We have previously pointed out¹ that a factor of two decrease in precipitation during the late glacial period at Dome C is consistent with the hypothesis that precipitation on the Antarctic plateau has been limited by the saturated water vapour pressure above the inversion layer¹², as estimated from ¹⁸O values in the ice. That this observation also holds at Vostok can be seen in Fig. 2, where a precipitation rate based on vapour pressure⁶ is compared with that estimated from the ¹⁰Be. In fact, the agreement of the two curves indicates that the inversion temperature may have been the dominant factor in determining precipitation rates on the Antarctic plateau during the past climatic cycle. This hypothesis is the basis for the adoption by Lorius *et al.*⁶ of a model where the past accumulation rate is assumed to be correlated with temperature, as deduced from oxygen isotope analysis. Several possible explanations why ¹⁰Be may be anti-correlated with precipitation rates in polar regions are discussed in ref. 9.

The chronology that follows from a reduced accumulation rate during the glacial period, together with a flow model described in ref. 6, is shown in Fig. 1. Although this timescale

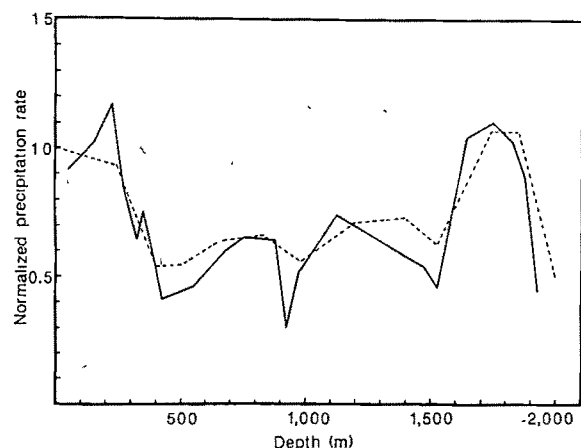


Fig. 2 Estimated precipitation rate (normalized to Holocene value) at Vostok in the past assuming a constant ^{10}Be deposition flux (solid line) and precipitation controlled by saturated water vapour pressure at temperature of inversion layer (dashed line, from ref. 6).

appears reasonable, and correlates with several other parameters⁶, it is obvious that a confirmation by direct measurement of the age of the ice at some point in the ice age is desirable. We hope to make such a measurement in the future using an accelerator mass spectrometry determination of the ^{14}C contained in the ice.

The only significant deviation between the ^{10}Be and ^{18}O curves of Fig. 1 is in the ^{10}Be measurement at 925 m. The ^{10}Be concentration in this sample is nearly 50% larger than any other, and does not correspond to the ^{18}O minimum. It is dangerous to construct any hypothesis on the basis of a single point, but it is conceivable that this sample may be showing enhanced ^{10}Be for reasons similar to that during the Maunder minimum, that is, decreased solar modulation. We plan to make more detailed measurements around this point.

In conclusion, although we do not exclude the possibility of production changes, the 'first order' cause of ^{10}Be concentration variations in Antarctic ice during the last climatic cycle seems to result from precipitation rate variations. ^{10}Be measurements may thus be a useful way of determining these precipitation rates and helping to establish chronologies. The present work suggests that the precipitation rate in the Antarctic during the last interglacial was similar to the Holocene, whereas during the ice age it was about $2 \times$ lower. The good correlation between ^{10}Be and temperatures deduced from oxygen isotopes also suggest that temperature has been the dominant factor controlling precipitation on the Antarctic plateau for at least the past ~ 150 kyr.

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Net flux of carbon dioxide from tropical forests in 1980

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Deforestation in the tropics is responsible for an annual net release of carbon to the atmosphere, estimated for 1980 at between 0.5 and 4.2×10^{15} g (refs 1–6). By comparison, the release of carbon from combustion of fossil fuels was 5.2×10^{15} g in 1980. The wide range of estimates for the tropical biota and soils has been due primarily to different estimates of the rate of deforestation^{2,7}. A recent assessment of the world's tropical forests by the Food and Agriculture Organization/UN Environment Program^{8,9} provides a comprehensive, country-by-country survey of deforestation in the late 1970s as well as estimates of the volumes of wood in tropical forests. The assessment thus provides an independent data base, perhaps the most reliable to date, from which terrestrial releases of carbon to the atmosphere can be calculated. Here we compare the FAO/UNEP survey with other sources of data on rates of deforestation and stocks of carbon in the tropics; then we present the calculated net flux of carbon between tropical forests and the atmosphere based on these different data; and, finally, we consider the accuracy of current estimates of the net biotic flux.

The FAO/UNEP's^{8,9} estimate of deforestation in the tropics is compared in Table 1 with estimates made by Myers^{10–12} and the FAO's Production Yearbook¹³. Comparison of the rates given by FAO/UNEP and the Production Yearbook assumes that deforestation of the open and closed forests of the former is equivalent to the net decrease of the forests and woodlands of the latter. Because the Production Yearbooks include fallow forests in their category of forest and woodland, while

Table 1 Annual rates of deforestation of tropical forests (1975–80) according to three sources (10^6 hectare yr^{-1})

	Closed and open forests		Closed forests	
	FAO/UNEP ⁸	Production Yearbook ¹³	FAO/UNEP ⁸	Myers ^{10,11*}
America	5 391	5 430	4 119	2.67
Africa	3 678	3 089	1 333	1.57
Asia	2 005	0 895	1 815	3.27
All tropics	11 074	9 414	7 267	7.51

* About 36% of the total conversion of humid forests to agriculture and pasture was from non-fallow forests^{10,11,14}. The rest was from fallow forests, not included in this table. About 20% of the conversion of forests due to fuelwood extraction was of non-fallow forests. These 0.5×10^6 hectare yr^{-1} were divided equally among regions

FAO/UNEP does not include the deforestation of fallow, the comparison is not strictly valid. Nevertheless, it shows agreement for the entire tropics within 8% of the mean (Table 1).

Comparison of Myers' estimate with that of FAO/UNEP is only possible for closed forests, because Myers did not include dry forests or woodlands in his survey. Furthermore, Myers included the clearing of fallow as well as primary forests. The primary forests of Myers correspond to the primary, secondary, and logged forests of FAO/UNEP. His secondary forests correspond to fallow forests in the terminology of FAO/UNEP. When Myers' estimates of total conversion are partitioned between primary and secondary forests on the basis of the distribution

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Table 2 The rates (10^6 hectare yr^{-1}) of conversion of fallow and non-fallow forests to permanently and temporarily cleared land; the rates are for 1980 and apply to closed forests of the humid tropics

	Non-fallow forests			Fallow forests			Total conversion
	Permanent	Temporary	Total	Permanent	Temporary	Total	
Pasture	0.7	0	0.7	1.3	0	1.3	2.0
Fuelwood	0.5	0	0.5	2.0	0	2.0	2.5
Agriculture	3.9	1.9	5.8	6.8	3.4	10.2	16.0
Total	5.1	1.9	7.0	10.1	3.4	13.5	20.5

Two and three significant figures are for clarity and consistency and do not indicate the level of accuracy. Myers¹¹ estimated that 90 and 50 million of the subsistence agriculturalists occupied fallow and non-fallow forests, respectively (Myers' terminology of primary and secondary forests is equivalent to the terminology of non-fallow and fallow forests used here). The annual rates of deforestation due to subsistence agriculture (16.0×10^6 hectare yr^{-1})¹² and to ranching (2.0×10^6 hectare yr^{-1}) were assumed to be distributed between non-fallow and fallow forests in proportion to these numbers of people. The proportions of non-fallow and fallow forests cleared as a result of fuelwood harvest (2.5×10^6 hectare yr^{-1}) were 20% and 80%, respectively¹⁴.

Table 3 Carbon in vegetation and soils of different land-use categories in the world's major tropical regions

Tropical region	Type of forest	Carbon in vegetation* (10^3 kg hectare ⁻¹)			Carbon in soils (10^3 kg hectare ⁻¹)		
		Undisturbed forest	Mature fallow	Agriculture	Undisturbed forest	Mature fallow	Agriculture
America	Moist	82 (176)	33 (70)	5	100	90	70
	Seasonal	85 (158)	34 (63)	5	100	90	70
	Dry	27 (27)	11 (11)	5	69	62	48
Africa	Moist	124 (210)	50 (84)	5	100	90	70
	Seasonal	62 (160)	25 (64)	5	100	90	70
	Dry	15 (90)	6 (36)	5	69	62	48
Asia	Moist	135 (250)	90 (90)	5	120	108	84
	Seasonal	90 (150)	50 (50)	5	80	72	56
	Dry	40 (60)	35 (35)	5	50	45	35

* Values outside parentheses are derived from volumes of growing stock^{8,9}. Values inside parentheses are based on direct measurements of carbon stocks¹⁷.

of subsistence agriculturalists^{11,14}, his rates of deforestation of primary forests agree well with the FAO/UNEP estimate for closed forests (Table 1). Agreement is within 3% of the mean for all the tropics, but within 60% of the mean for Asia. If Myers' estimates of deforestation apply strictly to tropical, moist, broad-leaved forests, his estimate is ~25% higher for the entire tropics than the FAO/UNEP estimate based on the same criteria¹⁴.

The major difference between the surveys of Myers and FAO/UNEP is not their estimates of current rates of deforestation, however, but their assumptions concerning the fate of fallow lands. The traditional form of agriculture in the tropics, shifting cultivation, has been largely replaced in recent years by a more permanent type of agriculture, referred to as "forest farming" by Myers¹⁰⁻¹² or as "sedentary shifting cultivation" by the US National Research Council (NRC)¹⁵. Even where the cultivators themselves are shifting, the land may remain permanently cleared by the arrival of new colonists or by the inability of forests to re-establish after intensive use of the land. The FAO/UNEP study, although recognizing the process, did not address the deforestation of forests that had been previously in agriculture (fallow forests) because such forests were not considered a timber resource. In the context of a carbon budget, however, the conversion of fallow forests to permanent agriculture is important because it reduces the amount of carbon stored on land. In the traditional form of shifting cultivation, the carbon released to the atmosphere on clearing is stored again during the re-establishment of forests in the fallow period. Over time, the net flux of carbon may be zero. If forests are eliminated permanently, however, the net flux is to the atmosphere.

The fraction of land cleared for agriculture that is permanent, as opposed to shifting, varies widely among regions: we estimate 66% for the tropics as a whole. This is a crude average which is based on estimates for individual countries and regions. Myers (unpublished data) estimates that the fraction of land cleared permanently may be as high as 95% in Brazil, and 90% in Columbia, Peru, Ecuador, and the Amazonian sector of Bolivia.

For all Latin America, however, an estimate of 85% was judged more appropriate because it accounts for the lower fraction of permanent conversions in Central America. In Asia, the fraction was estimated to be 70%, representative of a mean between high fractions where colonization of logged forests is common and low fractions where the more traditional shifting cultivation still predominates (for example, Papua New Guinea). In Africa, the overall average was estimated to be about 50%. It may be as low as 10% in Zaire but much higher in West Africa, especially since the drought in the Sahel has forced populations southwards into the forests. The fraction of clearing for subsistence agriculture estimated to result in the permanent clearing of forest was assumed to apply to the clearing of both fallow and non-fallow forests (Table 2).

The rates of temporary and permanent deforestation of non-fallow and fallow closed forests as derived from Myers' estimates¹⁰⁻¹² are shown in Table 2 and summarized in Fig. 1. Although Myers did not explicitly define these different pathways, the rates given in Table 2 and Fig. 1 are the only set of rates consistent with his data and with the fractions of temporary compared with permanent clearing given above. Deforestation of non-fallow closed forests (A in Fig. 1) was 7.0×10^6 hectare yr^{-1} . This rate includes the conversion of primary, secondary, and logged forests to both permanently cleared land (5.1×10^6 hectare) and to shifting cultivation (1.9×10^6 hectare yr^{-1}) (Table 2). The total is slightly lower than the one in Table 1 based on Myers' earlier estimates^{10,11}, and is close to the FAO/UNEP estimate of 7.3×10^6 hectare yr^{-1} . The breakdown between permanent and temporary deforestation differs from the FAO/UNEP estimate of 4.1×10^6 and 3.4×10^6 hectare of closed forest, respectively, but the difference between FAO/UNEP and Myers in this respect is not large.

The large difference is in their estimates of the change in fallow area. According to Myers' data 10.1×10^6 hectare of fallow forests are converted each year to permanently cleared land (Table 2). The net reduction in fallow (C-E in Fig. 1) is 8.2×10^6 hectare yr^{-1} , a reduction larger than the annual reduction in

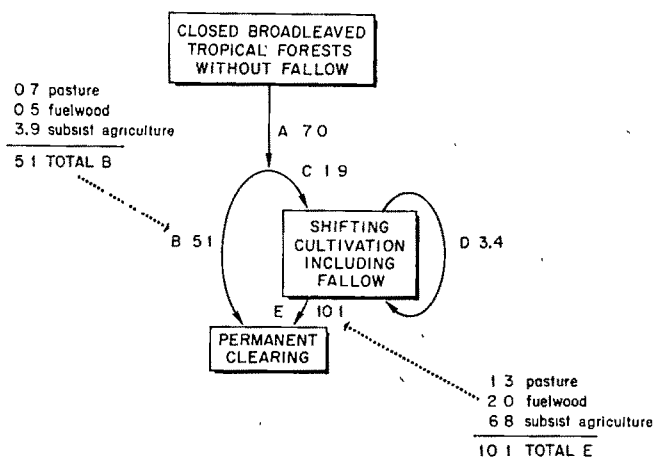


Fig. 1 Annual rates of conversion of tropical humid forests to other uses of land (10^6 hectare) based on data from refs 10–12, 14 and Myers (personal communication)

area of non-fallow forests. The FAO/UNEP study, in contrast, estimates that the area of fallow in closed forests is increasing annually at a rate of 3.4×10^6 hectare (ref. 9). If Myers is correct, a total of 15.2×10^6 hectare of forest land is being permanently cleared each year in the tropics, with 5.1×10^6 hectare coming from non-fallow forests and 10.1×10^6 hectare coming from fallow forests that were once part of the shifting cultivation cycle (Table 2, Fig. 1).

The second place where data from the FAO/UNEP survey contrast sharply with independent evidence is in growing stocks. Evidence from several reviews of terrestrial carbon stocks^{16–18} suggests that the estimates of growing stocks reported by FAO/UNEP are low by a factor of 2 when converted to carbon stocks (Table 3).

In this analysis, the volumes of growing stock reported country-by-country by FAO/UNEP were distributed among three types of forest in each region (America, Africa, and Asia) based on the density of growing stocks⁸. Mean volumes of wood, weighted by the area deforested⁸, were determined for forest type and region (Table 3). These mean volumes were converted to carbon with factors of 0.62 for the mean density of tropical wood¹⁹, 0.45 for the carbon content of wood, and 1.72 for those parts of the vegetation not included in commercial volume, such as twigs, leaves, roots, shrubs, and trees <10 cm in diameter²⁰. Similar conversion factors were derived independently by Brown and Lugo²¹.

The forests with high, medium, and low growing stocks in each region corresponded geographically to the evergreen equatorial forests, tropical seasonal forests, and tropical dry forests/woodlands, respectively, of Olson *et al.*¹⁸ Assignment of published estimates of biomass (cited in ref. 17) to these forest types on the basis of location yielded a second estimate of the carbon stocks for these forests. These estimates were about double those derived from the FAO/UNEP study. The same result was obtained by Brown and Lugo²¹ using more forest types.

The net release of carbon to the atmosphere as a result of clearing forests for permanent crops and pasture was calculated using a model described previously for global analyses^{2,22}. Logging and fuel-wood removals were incorporated in the analyses reported here by assigning masses of carbon simulating wood products to pools that oxidized at rates of 1/1, 1/10, or 1/100 yr^{-1} . This approach is consistent with the evidence that wood removals are most often coincident with deforestation. In Asia and Africa, logged forests are frequently settled by subsistence agriculturalists^{9–11}; in tropical America logging is generally carried out in forests destined for large development projects⁹. The model was expanded in this study to include the practice of shifting cultivation. Figure 2 shows the changes in carbon stocks that accompany shifting cultivation in the moist tropical forests of Asia (Table 3).

The model was used to calculate the net flux of carbon from

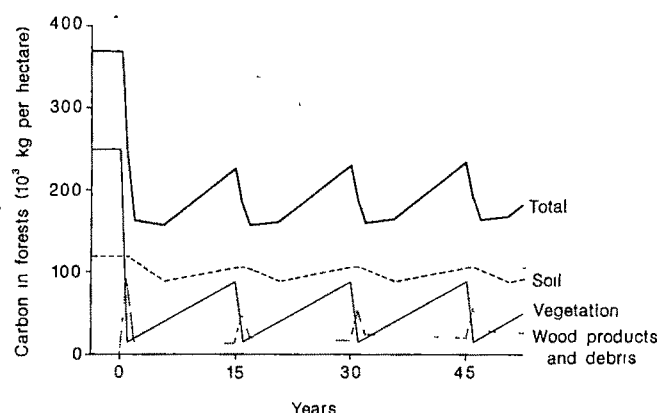


Fig. 2 Changes in the carbon content of vegetation, soils and pools of dead wood during three cycles of shifting cultivation.

tropical forests according to the three estimates of deforestation and the two estimates of carbon stocks described above. The carbon stocks of vegetation at the end of the fallow cycle (mature fallow) were determined from several sources^{23–25} as were the values for the carbon content of soils^{17,26,27} (Table 3). The conversion of forests to croplands and pasture was assumed to reduce the carbon content of the forest soils by 30% and 20%, respectively^{20,26}. Analyses were made for the three regions separately.

To allow a sufficient time for cohorts of the shifting cultivation model to stabilize, all modelling analyses were started in 1940. Because the only rates of deforestation given in the FAO/UNEP survey were average rates over the 5-yr period 1976–80, different assumptions about the rates of deforestation before 1980 were examined. Such differences affected the estimated flux of carbon in 1980 by less than 10%.

The net storage of carbon in forest plantations was not calculated in this analysis but is not thought to change the net flux significantly. For the tropics as a whole, the ratio of annual planting to deforestation is about 1:10 (ref. 9). In South-east Asia where the ratio is higher, the storage of carbon due to afforestation was <5% of the total net flux²⁰.

The net flux of carbon to the atmosphere in 1980 based on the FAO/UNEP estimate of deforestation of non-fallow forests in the tropics was about 1.4×10^{15} g C with the higher estimates of carbon stocks and 0.7×10^{15} g C with the lower estimates (Table 4). The three different estimates of deforestation gave remarkably similar estimates of flux for the tropics as a whole, reflecting similarities in the rates of deforestation for the entire tropics (Table 1). Within regions, however, different estimates of deforestation generated net fluxes that varied by about a factor of two. Summing the lowest and highest estimates of flux for each region gave ranges of 0.507 – 0.826×10^{15} g C, respectively, for the lower estimates of carbon stocks and 0.982 to 1.675×10^{15} g C for the higher estimates (Table 4).

The permanent deforestation of 10.1×10^6 hectare of fallow forests (Table 2, Fig. 1) was estimated to contribute another 0.4 to 0.8×10^{15} g C to the atmosphere in 1980 depending on the stocks of carbon assumed for fallow lands. For computation of

Table 4 Calculated net release of carbon to the atmosphere in 1980 (10^{15} g) from deforestation of non-fallow forests in the tropics (Table 1)

	FAO/UNEP	Myers	FAO
America	0.344 (0.549)	0.220 (0.400)	0.419 (0.783)
Africa	0.175 (0.390)	0.184 (0.406)	0.177 (0.394)
Asia	0.198 (0.362)	0.223 (0.486)	0.112 (0.190)
All tropics	0.717 (1.401)	0.627 (1.292)	0.708 (1.367)

Values in parentheses are based on calculations with higher stocks of carbon¹⁷. Other values are based on FAO/UNEP estimates of wood volumes converted to carbon stocks as described in text.

the loss of carbon associated with the conversion of fallow forest to permanent agriculture, the carbon content of the median aged cohort in the fallow cycle, rather than that of mature fallow, was used (Fig. 2). The resolution of the data did not warrant regional analyses, and the calculation was made for the tropics as a whole. The total net release of carbon from the tropics due to deforestation was thus estimated at between 0.9 and 2.5×10^{15} g C. Uncertainties in the rate of deforestation, in the stature of forests cleared, and in the length of time forests remain cleared contribute about equally to this range.

Both the range and the magnitude of the net flux of carbon in 1980 were lower in this analysis than previously reported by Houghton *et al.*² (1.3 – 4.2×10^{15} g C). Reduction of the upper end of the range resulted from a reinterpretation of Myers' estimate of deforestation. Houghton *et al.* assumed that two-thirds of Myers^{10,11} estimate for total conversion (22.5×10^6 hectare yr^{-1}) represented permanent deforestation, as Lanly and Gillis²⁸ had found in Latin American forests. As the analysis presented here reveals, the permanent conversion of non-fallow forests to other uses of land was only 7.0 – 7.5×10^6 hectare yr^{-1} in 1980 (Tables 1 and 2).

Data from the Production Yearbooks of the FAO were also used in a different way in this study from the earlier one. Houghton *et al.*² used changes in agricultural and grazing land to derive rates of deforestation. They distributed the total increase in agricultural area between both forest and non-forest systems, so that the decrease in area of forests was less than the increase in agricultural land. However, the loss of forests and woodlands has exceeded the growth of agricultural and grazing lands, according to the Production Yearbooks, and in the analysis presented here deforestation was determined from change in the area of forests (Table 1). The excess loss of forests has been to 'other land', a fourth category given by the Production Yearbooks. 'Other land' includes many types of land, the most important of which in this context is land that has lost its capacity to support either agriculture or forest. The carbon stocks of such lands are low.

The third analysis of Houghton *et al.*² based on population growth and per capita use of agricultural land gave a flux of carbon from the tropics in 1980 (2.1×10^{15} g C) within the range presented here despite a lower rate of deforestation. The net flux of carbon was lower in the present work for several reasons. The reduction of soil carbon following clearing of forests was 30% in this analysis (Table 3); in the earlier analysis it was 50%. The lower estimate was based on a more extensive review of literature^{20,26}. The estimates of the stocks of carbon in vegetation were also lower for Latin America and Africa. The use of carbon stocks derived from the FAO/UNEP study reduced these estimates still more. Finally, the harvest of wood in this analysis was coupled with the clearing of forests for agriculture^{9,10} and, therefore, accounted for a smaller net release of carbon than in the earlier analysis where harvest was treated independently of deforestation.

The range reported here (0.9 – 2.5×10^{15} g) is higher than the ranges found in recent studies by Detwiler *et al.* (personal communication) and Molofsky *et al.*³, 0.5 – 1.8×10^{15} g C and 0.6 – 1.1×10^{15} g C, respectively. Both studies were based on models similar to the one used here and both used the same FAO/UNEP study to provide one estimate of forest biomass and one estimate of deforestation. If the deforestation of fallow lands is ignored in the analysis presented here, the ranges found by all three studies are similar.

The agreement, however, is more a reflection of the data available and the similarity of methods of analysis than a measure of how accurately the rates of deforestation or the carbon stocks of vegetation and soil are known. If the estimates of deforestation shown in Table 1 (non-fallow forests only) are assumed to represent the best estimates, agreement seems to be within about 100%, although in Asia, and India in particular, there are differences greater than 100%.

The major discrepancy between the estimates of Myers and FAO/UNEP is the direction and rate of change in area of fallow.

The size of the discrepancy is dependent on the fraction of deforestation estimated by Myers to be permanent. Lower fractions would reduce the difference and reduce the release to 0.4 – 0.8×10^{15} g C yr^{-1} calculated to result from the elimination of fallow forests. Resolution of the difference probably requires the use of extant and future data from satellites. Monitoring tropical forests with Landsat can establish rates of deforestation and whether the deforestation is permanent or temporary^{7,2}. Landsat can also be used to establish the age, and indirectly the carbon stocks, of fallow forests regrowing on lands cleared since 1972.

The difference between estimates of carbon stocks based on measurements of biomass¹⁷ and those derived from estimates of wood volumes reported by FAO/UNEP^{8,21} (Table 3) affects the range of flux estimates about as much as the uncertainty in deforestation rates. It is not clear which of the two estimates is based on samples more representative of extant forests, and whether the lower stocks of carbon are a result of natural variation or a result of disturbance by man.

The latter distinction is important because analyses here and elsewhere^{2–6} use change in the area of forests to calculate changes in the amount of carbon stored on land. Reduction of carbon stocks within areas of forest have been ignored, largely because the process of degradation is gradual and not as readily documented as deforestation. Such degradation occurs throughout the tropics, however, as a result of logging, harvest of fuelwood, deliberate burning, and grazing^{8,9}. The extent of this degradation and the net release of carbon associated with it cannot be evaluated at present; the reduction per unit area varies considerably and the areas affected are large. The direction of the net flux of carbon is clear, however. It is from land to the atmosphere. Thus the net releases of carbon from biota and soils estimated here are probably underestimates.

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Entrainment and vertical transport of deep-ocean water by buoyant hydrothermal plumes

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Submarine hydrothermal vents produce effluent plumes in the water column which can be easily detected with tracers such as ^3He , manganese and methane¹⁻³. Comparison of the tracer concentrations and temperature anomalies in such plumes with direct measurements of the pure vent waters shows that the plumes are typically mixtures of 1 part vent water with 10^4 parts ambient sea water¹. However, the extent to which hydrothermal plumes entrain and transport sea water and contribute to deep-ocean mixing is not known. We present here hydrographic data collected in the vicinity of an active hydrothermal field on the Endeavour segment of the Juan de Fuca Ridge where a well-defined effluent layer resides in the water column ~200 m above the depth of the vent field. The temperature and salinity signature of this layer indicates that a small volume of hot vent water can be very efficient at entraining ambient sea water. Directly above the Endeavour vent field, the effluent layer is composed of 0.01% vent water, 30% ambient water normally found at that depth, and ~70% entrained water which has been transported from deeper in the water column.

As shown in Fig. 1, the Endeavour segment of the Juan de Fuca Ridge is a 170-km section of mid-ocean spreading centre bounded by the Cobb Offset in the south and the Sovanco Fracture Zone in the north. In 1984, two *Alvin* submersible expeditions to this area discovered and mapped an active hydrothermal system within the axial valley of the spreading centre^{4,5}. Although the exact extent of the hydrothermal zone is unknown, a large vent field at 2,200 m depth near 47°57' N, 129°06' W was mapped in detail by *Alvin*. The field covers an area 150 × 250 m and contains many 'black smokers' venting water at temperatures up to 400 °C (refs 5, 6). The hydrographic properties in the region were mapped by vertical profiling with a Neil Brown conductivity-temperature-depth (CTD) probe, and for near-field studies the CTD was mounted directly on the submersible during several dives⁷.

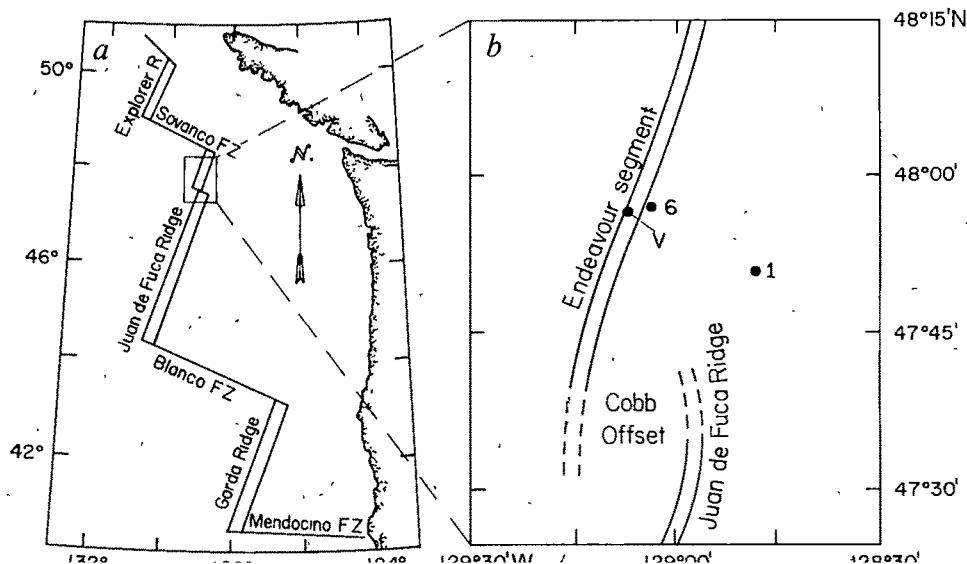
The effluent layer over the Endeavour segment was discovered with the striking profiles shown in Fig. 2, which were collected in 1983, 1 yr before the existence of hydrothermal venting was

confirmed by *Alvin*. Figure 2 shows CTD profiles of temperature, salinity and potential density at TT-175, Station 6, ~4.4 km east of the vent field. For comparison, a 'background' temperature profile is shown for TT-175, Station 1, ~26 km east of the vent field. Relative to this background profile, the Station 6 profile shows a clear temperature excess of ~0.050 °C over the interval 1,960–2,140 m depth. This feature is present to some degree in all the CTD profiles collected within 5–10 km of the vent area both in 1983 and 1984, and we conclude, therefore, that this hydrothermal layer is a long-lived feature of the water column in this region. The salinity and density profiles also reveal the existence of the effluent layer: the top of the layer coincides with a pronounced hump in the salinity, and the variation of density with depth within the layer is much smaller than above or below, indicating lower stability (lower Brunt-Vaisala frequency). Note that despite the dramatic temperature excursion, the layer is nonetheless stable, that is, the water in the layer has no tendency to rise or descend.

The distinguishing features of this layer are even more apparent in property-property plots of potential temperature, salinity and potential density as shown in Fig. 3. In the north-east Pacific, these three properties form mixing lines which are almost perfectly linear for water masses sampled below ~1,000 m depth. The injection of hydrothermal vent water is one process which violates this linear mixing relationship, and the anomalous properties of the layer are exhibited as clear deviations from the linear trend. We make the reasonable assumption that the density of the layer is nearly identical to that of the water which would reside at the same depth in the absence of hydrothermal activity. Figure 3 indicates, therefore, that the layer has an excess of temperature of ~0.050 °C, and excess salinity of ~0.007‰, relative to the predicted properties for water at that density. The excess temperature and salinity of the layer exactly compensate each other to produce a mixture of the appropriate density.

The excess temperature of the layer has obviously come from the hot vent water, and suggests that vent water averaging 300 °C has been diluted by a factor of ~7,000 before reaching the layer. However, the salinity excess cannot be attributed directly to the vent water, as the salinity of the vent water would have to be >60‰ to produce the salt anomaly in the effluent layer. The pure vent water samples collected by *Alvin* at this site have salinities quite close to that of ambient sea water⁸, and CTD data collected by *Alvin* in near-field buoyant plumes show no evidence of salinities higher than ambient (see Fig. 4). Therefore, we attribute the salinity excess in the effluent layer to the entrained water which has been transported vertically by ~200 m in the water column from a region of higher ambient salinity. Using the salinity difference between bottom water in the vicinity of the vent field (2,200-m depth) and water at the depth of the layer (2,000 m), and assuming all of the entrainment occurred

Fig. 1 *a*, Map of active spreading zones in the north-east Pacific. *b*, Detailed map of study area on Endeavour segment of the Juan de Fuca Ridge, showing the location of the hydrothermal vent field (V), and the hydrographic stations (1 and 6) occupied during expedition TT-175 in 1983.



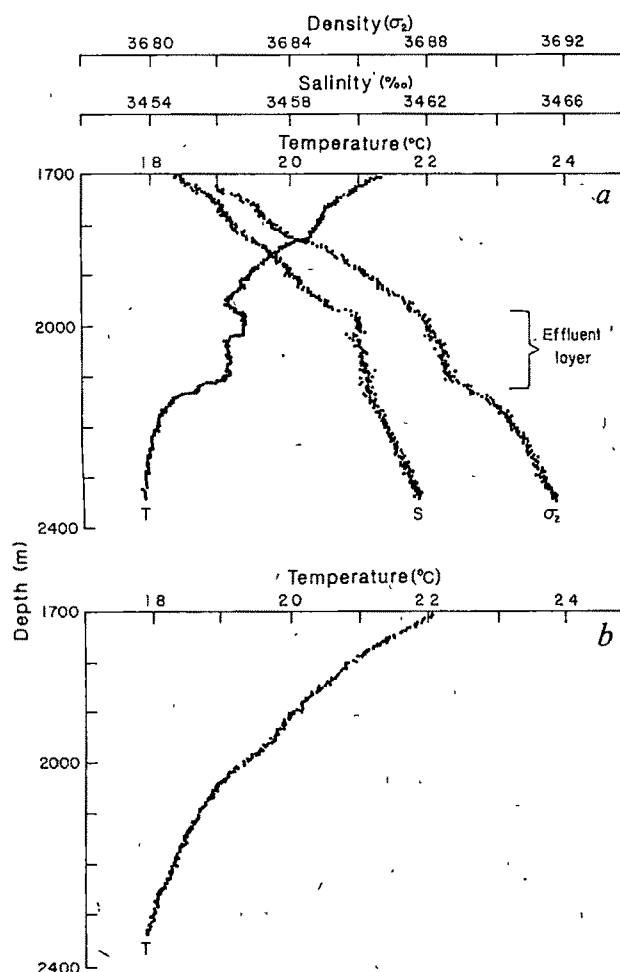


Fig. 2 a, Vertical profiles of temperature, salinity and potential density for Station 6, expedition TT-175, collected with a Neil Brown CTD in August 1983, at 47°57.5' N, 129°03.0' W, ~4.4 km east of the Endeavour hydrothermal vent field (see Fig. 1). Only the depth interval from 1,700 to 2,400 m is displayed. Potential density is expressed as σ_2 in units of % referenced to 2,000-dbar pressure. Brackets indicate the hydrothermal effluent layer which lies between 1,960 and 2,140 m depth. b, CTD temperature profile for Station 1, expedition TT-175 collected in August 1983, at 47°51.8' N, 128°47.7' W, ~26 km east of the hydrothermal vent field (Fig. 1). This serves as a 'background' profile, as it is largely unaffected by hydrothermal input.

at 2,200 m depth, we calculate that the layer must consist of 70% entrained water and only 30% water which would normally reside at 2,000 m depth. If we make the more realistic assumption that entrainment has occurred throughout the depth interval from 2,200 up to 2,000 m, then the proportion of entrained water in the layer is even higher.

Note that this entrained component cannot be detected with vent water tracers such as ^3He , and heat. Only a property such as salinity, which varies appreciably with depth in ambient sea water and has no signal in the vent fluids, can be used to estimate the extent of hydrothermal entrainment.

In addition to the stable effluent layer centred at 2,000 m depth, we were able to sample several unstable buoyant plumes with the CTD mounted on *Alvin*. One example is the ascent profile for dive 1451, shown in Fig. 4, in which the submarine passed through a plume ~0.10 °C above ambient shortly after leaving the Endeavour vent field. The potential density within this plume is lower than the overlying water, indicating that this is a buoyant water mass which is in the process of rising and entraining ambient water.

Fluid dynamical models of buoyant plumes in a stable stratified environment make predictions which can be compared with our observations. Using a buoyancy flux of $1.7 \times 10^7 \text{ cm}^4 \text{ s}^{-3}$, estimated for a single black smoker, and an ambient density

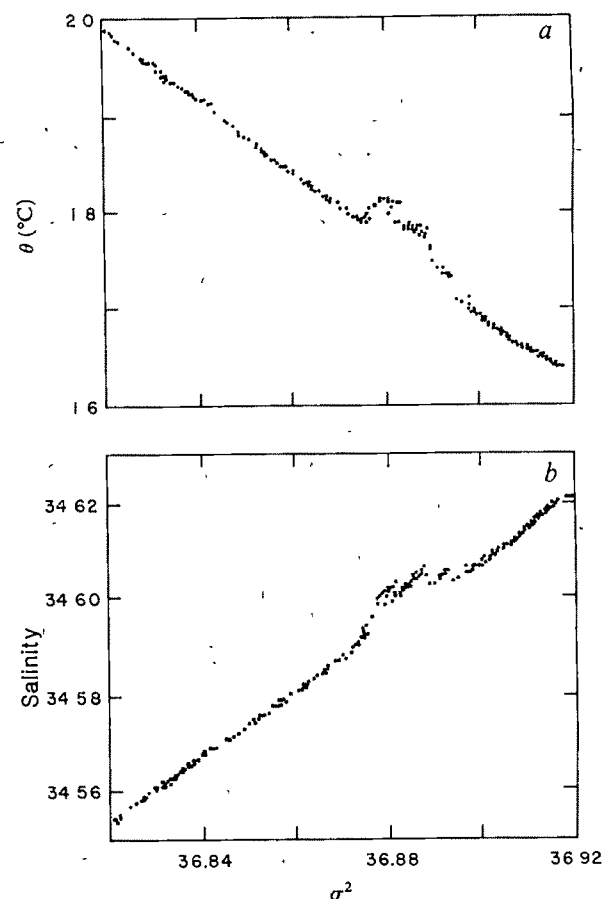


Fig. 3 Property-property plots of: a, potential temperature (θ) versus potential density (σ_2); and b, salinity versus potential density, for Station 6, expedition TT-175. The effluent layer (see Fig. 2) produces a marked excursion from the linear mixing trend which holds for ambient water masses unaffected by hydrothermal input.

gradient of $1.46 \times 10^{-6} \text{ s}^{-2}$ (Fig. 2a), the numerical model summarized by Turner⁹ predicts a maximum plume height of ~370 m and a ratio of entrained water to vent water of 3.4×10^4 at the top of the plume. Thus the model agrees roughly with our observations, which indicate a 200 m plume height and an entrainment ratio of $\sim 10^4$. The discrepancy suggests that although black smokers are the most visible type of discharge, they may not be the most typical. Thus a large fraction of the effluent layer may be a result of diffuse hydrothermal flows which individually produce buoyancy fluxes much smaller than that of a black smoker.

Stommel¹⁰ has calculated that the heat flux from hydrothermal vents should drive zonal circulation cells in the deep ocean that extend westwards from north-south trending ridges such as the East Pacific Rise and the Juan de Fuca Ridge. While Stommel assumed a plume entrainment factor of 10^3 and a plume temperature of 0.3 °C, our results indicate 10 times lower plume temperatures and 10 times more entrainment. We calculate that each calorie of hydrothermal heat should result in the vertical transport of ~25 cm³ of ambient water. A typical smoker contributes $\sim 10^7 \text{ cal s}^{-1}$, which is roughly equal to the average theoretical hydrothermal heat flux from a 1-km-long ridge segment spreading at 3 cm yr⁻¹ half rate^{11,12}. The 170-km-long Endeavour segment should introduce $2 \times 10^9 \text{ cal s}^{-1}$ and produce ~0.05 sverdrups ($5 \times 10^4 \text{ m}^3 \text{ s}^{-1}$) of vertical transport when the episodic hydrothermal activity is averaged over geological time scales. Thus the ~40 black smokers that are active at the Endeavour hydrothermal field are supplying a substantial fraction of the average hydrothermal heat flux for the 170-km long ridge segment.

The Endeavour vent field is only one of five or six active geothermal zones on the Juan de Fuca/Explorer ridge sys-

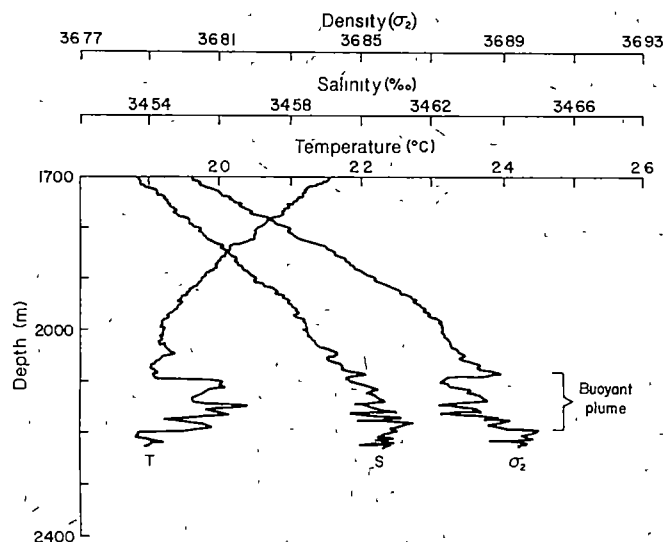


Fig. 4 Vertical profiles of temperature, salinity and potential density collected in September 1984, at the Endeavour hydrothermal vent field (see Fig. 1). The data were collected during the ascent of dive 1451 with the CTD mounted on the submersible *Alvin*. Shortly after leaving the bottom, the submarine passed through a hydrothermal plume $\sim 0.1^\circ\text{C}$ above the ambient temperature. The potential density signature shows that this was an unstable buoyant plume, quite different from the stable effluent layer shown in Figs 2 and 3.

tem^{4,5,13-17}. Applying our average hydrothermal heat flux calculation on a broader scale, the $\sim 760\text{-km}$ -long spreading system should produce ~ 0.2 sverdrups of vertical 'pumping' which, due to the considerable topography of the ridge crest, would deposit water onto several different density horizons. This transport is significant compared with the abyssal flows predicted for the deep north Pacific^{18,19}, suggesting that hydrothermal activity may influence the deep circulation patterns for a considerable distance from the Juan de Fuca Ridge axis, as predicted by Stommel¹⁰.

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Absolute motion and evolution of the Bouvet triple junction

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The absolute motion of the Bouvet triple junction has an important role in its mechanical evolution. At present, the relative velocity vectors of the South American, African and Antarctic plates form an obtuse isosceles triangle, and both the ridge-ridge-ridge (RRR) and ridge-transform-transform (RFF) configurations of the Bouvet are potentially stable. Sclater¹ demonstrated that the Bouvet twice alternated between RRR and RFF in the past 20 Myr. The reasons for these changes are unclear, but the analysis of the absolute velocities of the two configurations presented here provides some insight. The motion of the RRR configuration of the Bouvet, with respect to a hotspot reference frame, carries the junction to the north-west (343°) at 14.7 km Myr^{-1} , whereas the RFF form moves to the south-east (157°) at only 5.8 km Myr^{-1} . The RFF configuration is stable only when the relative velocity triangle is precisely isosceles. As the RFF junction migrates away from the isosceles line, it becomes increasingly unstable, until it is forced to switch to RRR. The RRR junction then drifts back to the isosceles line where it reverts to RFF.

The classic notion of triple junction stability introduced by McKenzie and Morgan² is based on constant plate velocities. McKenzie and Parker³, however, recognized that changes in relative velocities, which necessarily accompany the motion of a triple junction on the surface of the Earth, will affect its stability.

Minster and Jordan's AM1-2 absolute poles of rotation⁴ for the South American (SOAM), African (ARFC), and Antarctic (ANTA) plates, were utilized to calculate absolute plate velocities and to locate the locus of isosceles velocity triangles (Fig. 1) in the vicinity of the present Bouvet triple junction.

The actual Bouvet triple junction falls some 6° south of the calculated 'isosceles line'. This discrepancy probably reflects uncertainties in the absolute poles of the AM1-2 model because Sclater has shown that the relative velocities at the Bouvet junction form an isosceles triangle within observational error¹. To be consistent with the AM1-2 velocities, we assume here that the Bouvet triple junction is at 61.8°S ; 0°E .

Figure 2a is a velocity space diagram for the Bouvet triple junction illustrating both RRR and RFF configurations. The

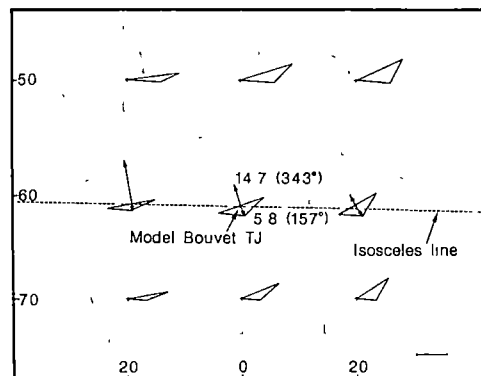


Fig. 1 The position and orientation of the isosceles line in the South Atlantic calculated from Minster and Jordan's AM1-2 absolute motion model⁴. The velocity of the triple junction in RRR and RFF modes is 14.7 km Myr^{-1} at 343° and 5.8 km Myr^{-1} at 157° , respectively. In RFF mode, the junction migrates south-east away from the isosceles line becoming increasingly unstable as the velocity triangle becomes distorted. Scale bar, 20 km Myr^{-1} .

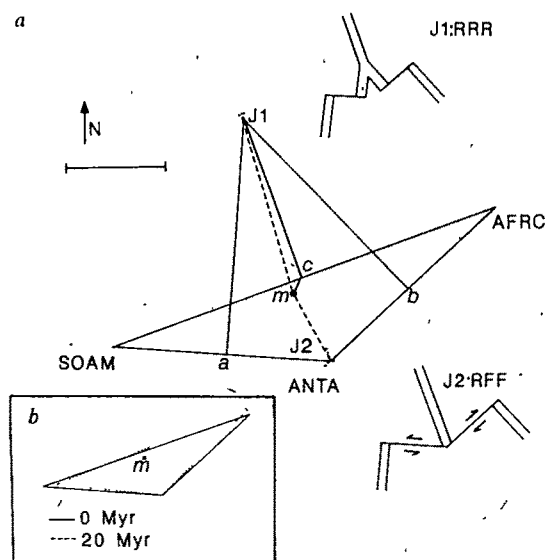


Fig. 2 *a*, A velocity-space diagram for the Bouvet triple junction with both RRR and RFF modes superimposed. In RRR mode vector, a -J1 and b -J1 are the growth rates of the SOAM-ANTA, AFRC-ANTA ridges and vector c -J1 is the rate that the SOAM-AFRC ridge is consumed. In RFF mode, vectors a -J2 and b -J2 are the rates of growth for the respective transforms, and vector c -J2 is the rate of ridge growth m is the location of the mantle reference frame. Vectors m -J1 and m -J2 are velocities of the triple junctions in RRR and RFF modes, respectively. Vector mc is the absolute velocity of the Mid-Atlantic Ridge. Two insets illustrate the direct space configurations. *b*, Changes in the shape of the relative velocity triangle which accompany the migration of a RFF junction away from the isosceles line. The rotation of the ANTA-AFRC and ANTA-SOAM vectors are much exaggerated to illustrate the fact that the original transforms become 'leaky'. Scale bar, 8 km Myr^{-1} .

RFF junction is stable only when the relative velocity triangle is isosceles. The vectors a -J2 and b -J2 are the rates of growth of the paired transforms which form when the junction is RFF. In the RRR mode, vectors a -J1 and b -J1 represent the growth rates of the SOAM-ANTA, AFRC-ANTA ridges. Vector c -J1 is the rate that the SOAM-AFRC ridge is consumed. The absolute velocities of the RRR and RFF junctions are vectors m -J1 and m -J2 respectively; m denotes the velocity of a mantle reference frame. Triple junction velocity vectors along the isosceles line are shown in Fig. 1. The Bouvet triple junction in

RRR mode has an absolute velocity of 14.7 km Myr^{-1} at 343° . When RFF, the junction migrates at 5.8 km yr^{-1} towards 157° . The orientation of these vectors change systematically along the isosceles line although they are oriented consistently in directions opposite one another.

Both the Conrad and Bouvet fracture zones are $\sim 150 \text{ km}$ in length¹. Assuming that the absolute velocities of the three plates have not changed significantly during the past 30 Myr, their rate of growth would have been 8.1 km Myr^{-1} (vectors a -J2 and b -J2, Fig. 2). This would require that the Bouvet junction remained in the RFF mode for 18.5 Myr. Using this and other information derived from the AM1-2 model, we can infer the following history for the Bouvet junction from the inception of the paired Bouvet and Conrad fracture zones.

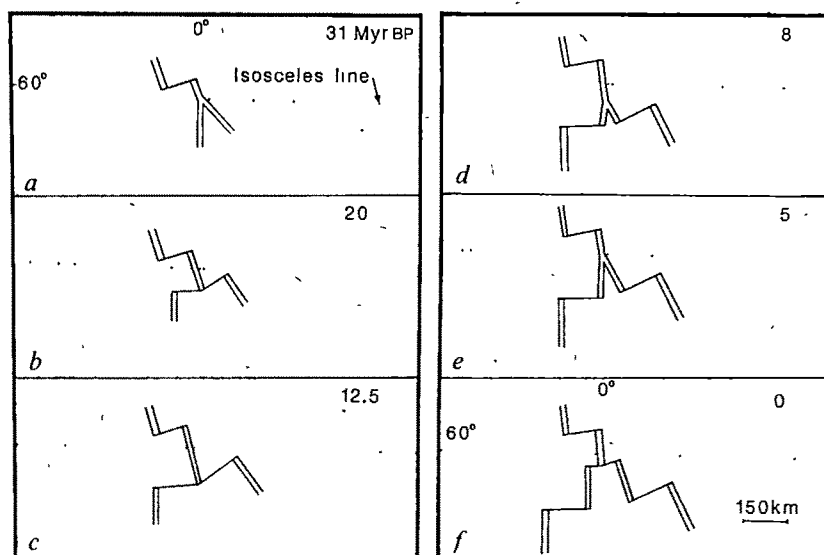
Sometime before 31 Myr BP, a RRR version of the Bouvet junction existed south of its present location. Its absolute velocity carried it northwards at 14.7 km Myr^{-1} . At 31 Myr BP the junction intersected the isosceles line and changed to RFF mode (Fig. 3*a*). In RFF mode (Fig. 3*b*), the junction migrated with a velocity of 5.8 km Myr^{-1} at 157° away from the isosceles line until 12.5 Myr BP when it reverted to its old RRR configuration (Fig. 3*c*). During the RFF episode, the SOAM-AFRC ridge grew 120 km and both the Conrad and Bouvet transforms were generated. As RRR, the junction then drifted back towards the north-west (343°) consuming the SOAM-AFRC ridge and lengthening the SOAM-ANTA, AFRC-ANTA ridges in the process (Fig. 3*d*). Finally, 5 Myr ago, the junction arrived in the vicinity of the isosceles line (Fig. 3*e*) and switched back to RFF. Since then, the junction has been moving south-east away from the isosceles line in its present RFF configuration (Fig. 3*f*).

The inferred sequence of events is similar to the history of the junction deduced by Sclater *et al.*, from magnetic data¹. The timing of the configuration changes are, however, slightly different. Magnetics date the first RRR to RFF transition at 10 Myr BP and the second at 5 Myr BP. These discrepancies reflect either the inaccuracy of the absolute motion model or suggest that the plate motions have changed slightly during the past 30 Myr. Nonetheless, the overall agreement supports the idea that the minor changes in relative velocities which accompany junction migration can have a significant role in its evolution.

Several questions remain. For example, the RFF configuration of the Bouvet junction should be stable only on the isosceles line. Its migration away from the isosceles line would render it immediately unstable. Yet it seems to have survived for at least 18.5 Myr (31–12.5 Myr BP) in RFF mode. Why?

The reasons behind its persistence may lie in the nature of the changes in plate velocities which accompany its south-east migration (Fig. 2*b*). The rotations of the relative velocities are

Fig. 3 A model of evolution for the Bouvet triple junction based on the AM1-2 absolute motion model¹ and the length of the Bouvet and Conrad fracture zones. At the isosceles line (*a*) (31 Myr BP) the junction switched from RRR to a more geometrically stable RFF mode. As it migrated southeastwards (*b*) (20 Myr BP) the junction generated the paired Conrad and Bouvet transforms and extended the length of the Mid-Atlantic Ridge. In *c* (12.5 Myr BP), the transforms reached their final length of 150 km, and the junction switched back to RRR mode and began migrating in a northwesterly direction. As RRR (*d*), the junction drifted back towards the isosceles line. In *e*, the junction intersected the isosceles line and began generating new paired transforms until the present (*f*).



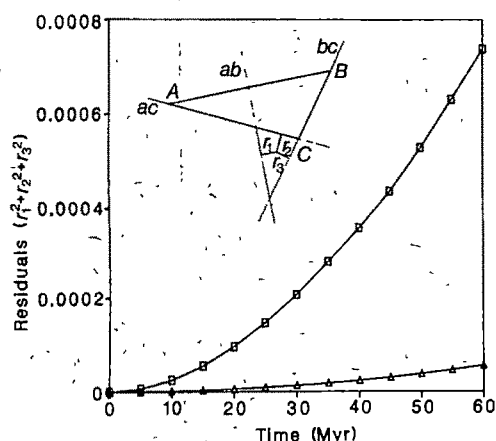


Fig. 4 Changes in the degree of geometric instability (residuals) as the RRR ($\square$) and RFF ($\triangle$) configuration of the Bouvet triple junction drifts away from the isosceles line. The orientations of the boundaries are assumed to be fixed, and spreading is assumed to be symmetrical. Instability arises because the relative velocities change with time. The RFF configuration is least affected by small changes in the velocity triangle, and it seems to be the preferred configuration in the vicinity of the isosceles line. The inset illustrates the geometric significance of the residuals.

such that the growing transforms develop a small component of extension. In other words, they become progressively 'leaky'. The actual rotations amount to only 1° over a period of 20 Myr, but this may be sufficient to make them mechanically feasible.

When the triple junction finally changes to RRR configuration, the newly generated slow spreading ridge segments must undergo rotation as the relative action vector changes during north-west migration. The MAR must also undergo a change in the spreading direction (Fig. 2b). This rotation is possibly accommodated by the generation of secondary transform faults along these ridge segments.

Questions also surround the reason why the junction switches from a RRR mode to RFF in the vicinity of the isosceles line. A partial answer may be suggested by the relative geometric instabilities of the two configurations. The location of the triple junction in two-dimensional velocity space is given by a set of three equations involving the three-plate velocities and boundary orientations. If these equations have a unique solution, the junction is stable in the sense of McKenzie and Morgan² and the velocity of the triple junction is well defined. If no solution exists, the triple junction is unstable. We suggest that the residuals of a least-squares fit in the case of an unstable junction can be used as a measure of the degree of geometric instability (see inset, Fig. 4).

For the Bouvet junction, the residuals for the RRR and RFF modes are plotted in Fig. 4 as a function of their travel time away from the isosceles line. In these calculations, the boundary orientations are fixed, and the spreading is considered to remain symmetrical. The residuals increase for RRR and RFF modes as the junction migrates. However, the instability of the RFF junction builds up much more slowly than RRR configuration. In the vicinity of the isosceles line (the origin of Fig. 4), the RFF junction is less susceptible to changes in the relative velocities perhaps explaining why it is the preferred state.

We conclude that the Bouvet triple junction is trapped in the vicinity of an isosceles line in the South Atlantic. Along this locus, the junction can exist in either RRR or RFF configuration, although RFF seems to be more stable. As the absolute motion of the RFF junction carries it away from this locus, it eventually becomes mechanically unstable and switches to a RRR configuration. The RRR junction has an absolute motion which drives it back to the isosceles line where it reverts to its original RFF configuration. Because the orientations of the absolute triple junction trajectories straddle the isosceles line, the Bouvet junction is destined to alternate between episodes of RRR and RFF as long as the absolute plate velocities remain unchanged:

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Geometry of propagating continental rifts

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Reconstruction of past plate configurations through palinspastic restoration of continental margins¹⁻⁴ requires a detailed knowledge of the geometries of horizontal continental extension. Extension is often restricted to discrete, linear zones termed rifts^{5,6}, and rift models that invoke propagating vertical dykes and cracks⁷⁻¹⁰ are useful for assessing the relative significance and timing of uplift, subsidence and extension in the tectonic evolution of rift basins. However, many models do not adequately account for the observed asymmetry of rifts. I describe here the general three-dimensional character of young and aborted continental rifts, which can be used to derive a structural model for the propagation of rifts in continental lithosphere. The rifts become asymmetric as a consequence of the role played by low-angle normal faults in the overall rift geometry.

Continental rifts are typically a few tens of kilometres in width, and several tens to a few hundred kilometres in length (Figs 1, 2). That these rifts are in some cases precursors to the complete rupturing of continental lithospheric plates seems to

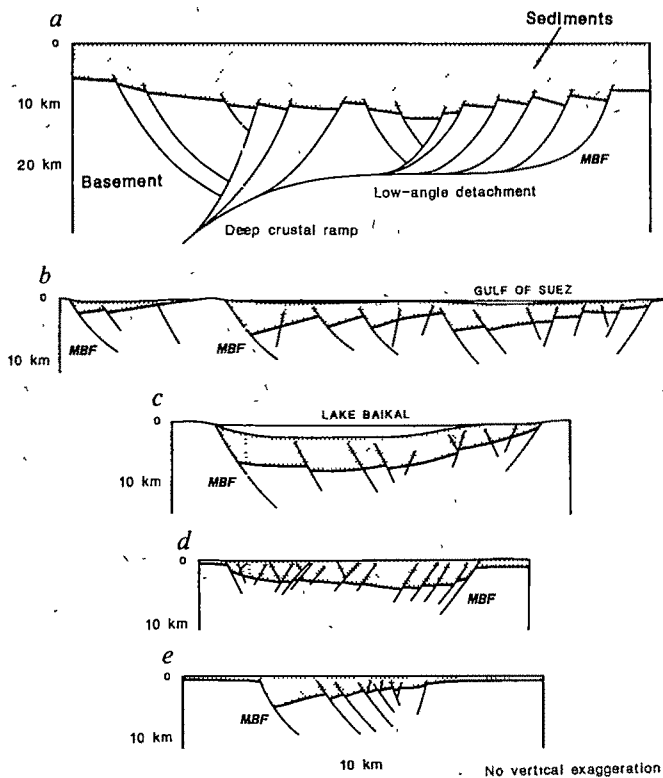


Fig. 1 Asymmetry of continental rifts in cross-section. Rifts commonly show half-graben-like forms in cross-sections taken normal to their long axes, with most basin relief generated by a single rift bounding fault (main bounding fault, MBF), or a system of a few main faults, which are inferred to bottom out to a low-angle detachment surface. *a*, Central Graben, North Sea, section (a submerged continental rift) is an approximate depth conversion of a time section presented by Gibbs²³. *b*, Section crossing southern Gulf of Suez, constructed from industry well and seismic data. *c*, Baikal Rift, sediment thicknesses from ref. 43, but internal faulting is largely schematic. *d*, Rhine Graben, from ref. 20. *e*, White Nile Rift, in part based on the gravity work of Browne *et al.*¹⁹.

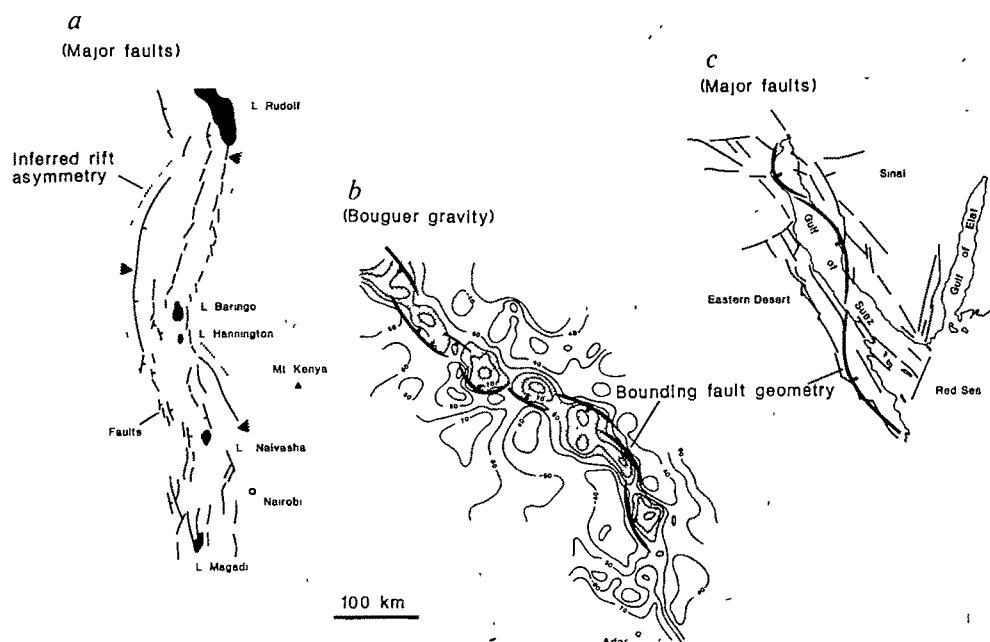


Fig. 2 Geometry of continental rifts in plan view. The major faults of continental rifts follow curvilinear patterns in map view, defining a sub-basin geometry that repeats generally every 50–150 km. Similar curvature is seen in the bounding faults of the Basin and Range Province⁴⁴. The asymmetry of the rifts in cross-section commonly reverses at each successive sub-basin, although not in every case. *a*, Gregory Rift, Kenya. Surface traces of major faults are from ref. 45. *b*, White Nile Rift, Sudan. Gravity data are from ref. 19. *c*, Suez Rift, Middle East. Surface faulting from ref. 17. Sub-basin geometry in the Gregory Rift (*a*) is more complex than portrayed here.

be well established by studies of the African–Arabian plate boundary (Red Sea and related rifts)^{11–15}. At the other extreme, continental extension may be distributed over broad zones, measuring hundreds of kilometres in each horizontal direction, as in the Basin and Range of the western United States. Whether the Basin and Range structure evolves from a few, initially discrete rifts, and whether it can eventually lead to continental breakup, are unanswered fundamental questions of continental tectonics.

Continental rifts are strongly asymmetrical in cross-sections normal to their long axis^{12,16} (Fig. 1). This asymmetry is evident in gravity-field gradients^{17–19}, relative rift shoulder elevations, sediment isopach maps^{20,21} and seismic reflection profiles^{22,23}. Meinesz²⁴ suggested that rifts would develop an initial asymmetry corresponding to the first formed bounding fault. As crustal flexure continued, the opposing fault breaks to produce a symmetrical graben form. Most models of rift formation^{8,12,25–30} have envisioned continental rifting, and hence the development of continental margins^{1,2,31} as essentially a symmetrical process at the scale of the lithospheric plate.

Many workers have suggested that the main faults of rifts pass completely through the crust to a zone of structural decoupling^{27,32,33} (Fig. 3). Recent geological and geophysical studies indicate that most of the extension within rifts and the larger Basin and Range framework is accommodated through displacement on low-angle normal faults^{22,23,24,29}. If the bounding faults of rifts link into such structures, and these fault systems cut entirely through the crust or lithosphere³⁴, then an asymmetrical rift structure would be expected. A symmetrical

arrangement of opposing low-angle detachments is untenable, as movement on one would offset the other, probably leading to the locking of one of the detachments.

Several geometries have been proposed for the detachments that are thought to underlie rifts. Wernicke³⁴ has suggested that these faults or fault zones are essentially planar on a regional scale. Alternatively, they may bottom out at a major crustal discontinuity such as the brittle–ductile transition³¹, and then plunge downwards at deep crustal ramps²³. Similarly, great diversity is observed in the internal fault patterns of rifts. Both planar fault arrays and curved (listric) fans are encountered in continental rifts^{35,36}. Differential rotation across larger rift faults, however, commonly indicates that they define a listric system. Listric faults curve in plan view as well as cross-section, but only towards the hanging wall block. Curvature towards the footwall is not kinematically viable. This effectively limits the horizontal extent of a single rift bounding fault (or system of parallel faults) to a few tens of kilometres (Fig. 2). Detailed seismic surveys reveal that rifts are typically broken into structurally-coherent compartments by transverse features^{31,37} analogous to lateral and oblique ramps in contractional tectonic settings. These structures referred to as ‘transfer faults’²³, are a syn-rift feature, although they may inherit a pre-existing structural grain.

On a larger scale, rifts also break up into sub-basins, which may form as isolated depositional systems (Fig. 2). Sub-basin boundaries may be defined by major transfer faults, pre-existing crustal discontinuities, or in some cases by younger strike-slip faults which simply offset an older rift. Significantly, rift asym-

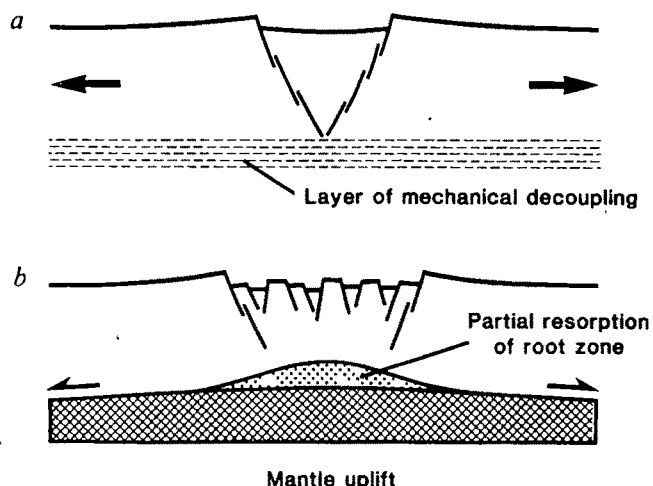
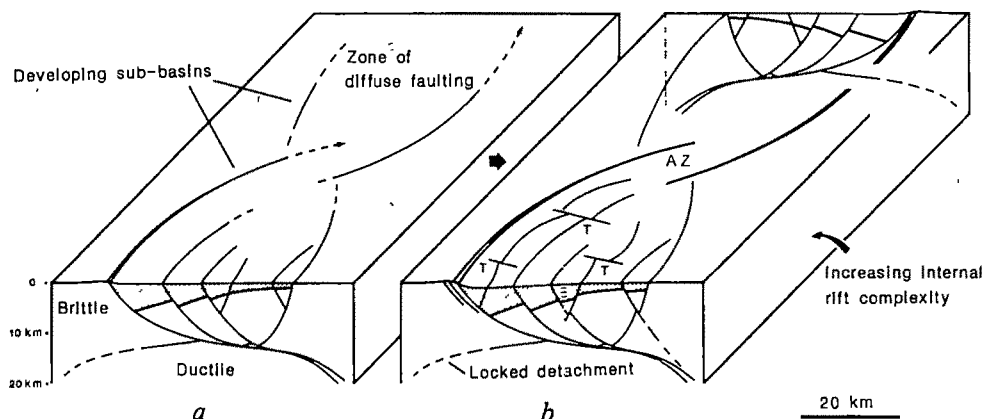


Fig. 3 Classical rift model of a brittle upper crust deforming above a ductile zone of mechanical decoupling (after ref. 30). The principal structural form of the rift itself is that of a symmetric graben, defining a triangular wedge-block at depth.

Fig. 4 Proposed model for the propagation of continental rifts. Upper crustal extension may initiate as a broad zone of diffuse faulting (a), but quickly evolves to a system composed of a few main listric faults (and perhaps in some cases planar fault-bounded blocks³⁵) above two oppositely-directed detachments. For both detachments to remain operative, their deeper sections would need to be repeatedly recut, due to their mutually offsetting geometry. This rarely occurs, judging from the observed asymmetry of most continental rifts (Fig. 1), and one detachment locks. The active detachment propagates along the rift axis (towards the pole of opening), but curves inwards to form a large-scale scoop-like structure⁴⁴. A theoretical treatment of this fault propagation would have to consider how this entire structure evolves—both the growth of the near-surface high-angle faults and the lateral propagation of the shallow-dipping detachments. Eventually the curving, active listric system departs enough from the overall rift trend to favour a new detachment system, which links to the old at a complex area referred to by Derksen and others³⁹ as an 'accommodation zone' (AZ). Again, opposing detachments may initially form, and the advantage may go to the detachment of opposite polarity. In this case, a reversal of rift asymmetry occurs, with greater down-faulting and sedimentation adjacent to the new main bounding fault (b). Detachment systems may overlap or merge at accommodation zones in a variety of configurations, but in the plan view shown here, a cross-section normal to the rift axis at the accommodation zone would actually appear to be a symmetric graben in form³⁹. The important cross-faults in rifts referred to by Gibbs²³ as 'transfer faults' (T) are kinematically similar to accommodation zones, on a smaller scale.



metry frequently reverses at sub-basin boundaries, as was early documented for the Gulf of Suez Rift³⁸ (Fig. 2). The change in asymmetry between adjacent rift sub-basins strongly suggests that the underlying low-angle detachment system also changes polarity. The area of asymmetry reversal was first termed a "hinge zone" by Moustafa³⁸ for the associated regional dip reversal on rotated fault blocks. As asymmetry remains constant at some sub-basin boundaries, with only an offset in the rift bounding fault (Fig. 2), these major crustal structures have been more appropriately referred to as "accommodation zones"³⁹.

Young rifts, and those that failed at an early stage of continental extension, can then be thought of as large-scale half graben (mega-half graben of Cohen⁴⁰), which flip more or less regularly along the axis of the rift (Figs 1, 2). When rifting is first initiated, two opposing low-angle detachments may develop simultaneously, and propagate in the direction of the pole of opening for the two diverging continental masses (Fig. 4). One of the detachment systems soon locks, and the half-graben form develops. At some point along the incipient rift zone, the opposing detachment gains the initial advantage, and a reversal of asymmetry is forced on the bounding fault system. There appears to be some degree of regularity in this process (Fig. 2), suggesting that the lateral limits on the dimensions of listric normal faults may, in part, control the spacing of accommodation zones. Extension across the rift zone and concomitant lithospheric thinning eventually become large enough to allow complete decoupling of the two now distinct lithospheric plates and the formation of new oceanic crust. At this point, the fault systems beneath opposite sides of the rift also become isolated and develop independent fault geometries.

Recent models for the propagation of continental rifts have attempted to assess the significance of early continental extension in the development of passive continental margins, and how that extension affects plate reconstructions¹⁻⁴. Asymmetries inherent in the rift scenario described above may be reflected in the structural and stratigraphical record of rifts long after oceanic crust first appears. In particular, a geoseismic model, stratigraphical column or thermal maturation curve derived at one point on a passive margin may not accurately apply to rocks a few tens of kilometres along strike, especially with respect to the initial rift sequence. Propagating oceanic rifts^{41,42}, where rift boundaries are almost immediately structurally decoupled, and propagating continental rifts, are undoubtedly quite disparate structural phenomena.

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Three distinct types of dynamic behaviour shown by a single planktonic system

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A wide range of dynamic behaviour has been uncovered by studying disparate predator-prey systems¹, ranging over population stability, cycles and non-cyclic fluctuations. We know of no instance, however, in which qualitatively different dynamics have been found in the same natural predator-prey populations at different times, or in a given predator-prey system found in different but similar communities. Mathematical models of predator-prey systems, by contrast, predict that any particular system may exhibit the whole range of dynamics simply as a result of changes in rate parameters, such as those that might occur at different temperatures. Indeed, May raises the possibility that different *Daphnia*-algal dynamics in the laboratory are caused by differences in the relative values of time lags and rates of increase at different temperatures². Therefore, to produce the different dynamics, the models need not undergo any change in structure, such as might occur in different systems or groups of organisms. Here we show that field populations of a predator, the zooplanktonic herbivore *Daphnia*, and its algal prey exhibit three qualitatively different types of dynamic behaviour. This finding supports the basic premise that different dynamics can result from quantitative differences within a constant structural framework, and hence the hypothesis that variations in dynamics between systems can be explained in the same way as differences within a single system.

Populations of *Daphnia* have been raised in the laboratory under constant additions of food at fixed 2-4-day intervals³⁻⁸. These populations are strictly food-limited, their average density being a linear function of the rate of food supply³. One outcome of these studies is sustained fluctuations in density that are sufficiently regular to qualify as cycles³⁻⁶, with a period of 20-40 days. As the rate of addition of algal food is fixed and, by design, the algae cannot grow during these experiments, the oscillations are a property of *Daphnia* demography; they are single-species rather than predator-prey (or herbivore-plant) oscillations. A second outcome is damped oscillations and a third is the absence of oscillation^{7,8}.

This impressive range of dynamic behaviour in *Daphnia*, despite constant conditions and fixed food supply, led us to survey the published studies of field population dynamics of *Daphnia*, concentrating on those for which data are also available on phytoplankton fluctuations. We restrict ourselves here to discussing the dynamics of 17 studies drawn from a wide diversity of freshwater habitats⁹⁻²³, and include our own data from two experimental populations. We have excluded studies in which competition between congeneric species of *Daphnia*²⁴⁻²⁶ or fish predation²⁷⁻³⁰ appear to be important factors in the dynamics (we will examine the effects of these factors elsewhere). We have not included three examples from very cold lakes in which *Daphnia* has only about one generation a year³¹⁻³³, because the longest of these studies covers only two generations.

An implicit assumption is that the *Daphnia* and the phytoplankton populations in these different habitats have sufficiently similar biology for them to be 'structurally' similar (that is, describable by the same general model), although their parameter values no doubt differ. This is reasonable for *Daphnia* species, which are basically larger or smaller versions of a single template. The phytoplankton in any particular habitat are made up of many species, and the species composition will vary from one habitat to another. Clearly, we cannot be certain that these collections all have qualitatively similar features. The phytoplankton are more homogeneous in important features, however, than their diverse taxonomy might suggest. In most temperate

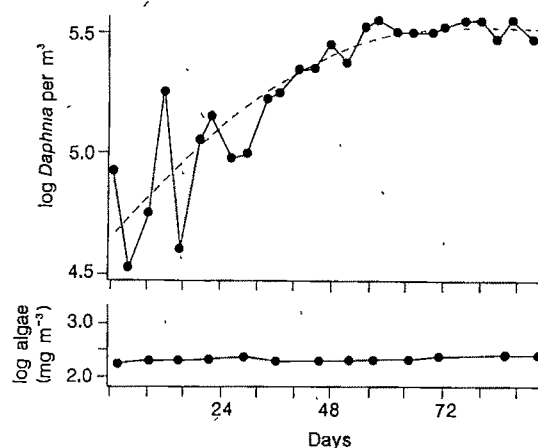


Fig. 1 An example of class 1 dynamics in which $\log_{10}$ *Daphnia pulex* Leydig abundance per m^3 and $\log_{10}$ algal biomass (mg m^{-3}) are stable. The dashed curve is a second-order seasonal trend fitted to $\log_{10}$ *Daphnia* density by least-squares regression analysis ($r^2 = 0.81$, $P < 0.001$). *Daphnia* density deviates little from this seasonal trend; the average residual deviation is $< 15\%$ of the trend value of the population. The experiment was carried out in 1,200-l stock tanks (described elsewhere⁴¹) on the campus of the University of California at Santa Barbara over a 4-month period between June and September (water temperature was 22-26 °C). These tanks represent a 'natural' habitat in the ranch country of southern California⁴². *Daphnia* populations were sampled every 3-4 days in duplicate (500-ml) samples that were counted in their entirety. Algal biomass was estimated as chlorophyll *a* and converted to cell volume.

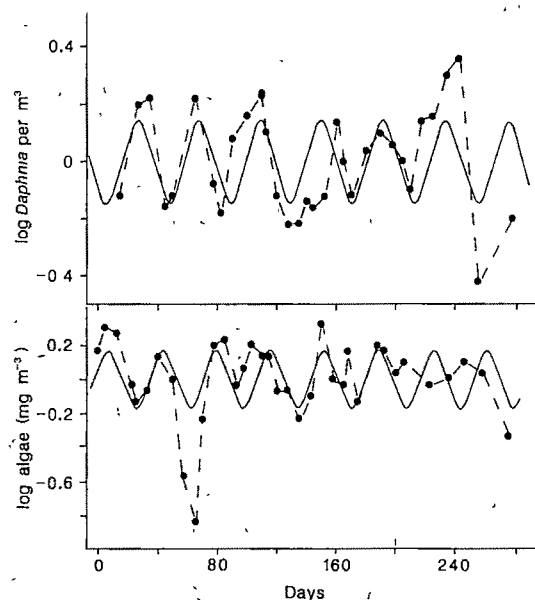


Fig. 2 An example of class 2 dynamics in which *Daphnia* and algae appear to exhibit predator-prey oscillations. These data are from ref. 14 (Table 1). The points joined by the dashed line are the residual deviations from (logarithmic) seasonal trends and the solid line is the sine function fitted to these data (Table 1).

zone lakes, a major proportion of algal biomass is $< 75 \mu\text{m}$, of which *Daphnia* can eat those up to $50 \mu\text{m}$. Indeed, for *Daphnia*, size is a more important feature than taxonomy in distinguishing algae. Edible species vary in growth rate, and perhaps also in nutritive quality, but these variations affect parameters and not structural aspects of the model.

Visual inspection of the data suggested that populations fall into three classes. (1) Stable driven populations. Algal population density is low, with sometimes a peak in autumn in response to an increase in nutrients. *Daphnia* populations usually follow the same pattern, apparently typically being driven by the algae, which in turn are driven by slowly changing environmental factors. *Daphnia* are apparently stable, as their density tracks

Table 1 Results of the analyses of *Daphnia* and algal field populations for the presence of cycles

Ref.	No. of observations (algae/ <i>Daphnia</i>)	Mean	Period (days) 95% c.l.†	P
Class 1				
9,10 (1978)*	49/24	None/none	NA/NA	NA/NA
11	20/15	None/none	NA/NA	NA/NA
12	19/24	213/none	185–243/NA	<0.0001/NA
13	19/29	None/none	NA/NA	NA/NA
Tank experiment	13/27	None/none	NA/NA	NA/NA
Class 2				
14	36/32	36/41	35–38/38–43	<0.05/<0.05
10,15 (1979)*	57/28	39/47	36–41/44–50	<0.05/<0.0001
10,15 (1980)*	23/24	37/45	32–42/41–50	<0.0001/<0.1
16	31/57	47/45	44–50/43–47	<0.05/<0.05
17	25/22	40/28	36–43/26–30	<0.05/<0.05
18	23/50	32/31	29–34/27–34	<0.1/<0.0001
19	22/22	37/36	32–43/32–41	0.0001/NS
20	22/22	32/25	30–33/24–26	<0.05/<0.05
21	26/26	40/36	30–50/29–42	<0.0001/<0.0001
22	24/24	32/36	28–35/29–42	NS/NS
Class 3				
Tank experiment	13/27	None/23	NA/22–25	NA/<0.0001
23	24/24	None/42	NA/38–46	NA/<0.05

A dominant feature of all natural systems is a spring peak and decline in both algae and *Daphnia*, driven by the spring increase in nutrient and light levels, and rising temperature. Our analyses covered the period following this decline. Given the observed range of periods in cycles in *Daphnia* laboratory populations, we did not look for cycles with a period shorter than 20 days; in most cases it was impossible to detect shorter periods given the frequency of sampling in the published studies. Standard time-series analysis was not possible because the data sets were too short, sampling periods were not regular and the two populations were not sampled at the same frequency. Instead, abundances were transformed to logarithms, seasonal trends where present were removed by fitting linear or second-order regressions³⁹. Populations were considered stable (no cycle present) if the average standard deviation of the logarithm of their densities was <20% of the mean logarithmic density. This percentage is a realistic estimate of sampling variability to be found in such systems, and provides an objective criterion for deciding if a population belongs to class 1. If the average standard deviation of the logarithmic residuals was greater than 20%, the residuals were subjected to Kendall and Stuart's turning point test to detect cycles⁴⁰, and sine functions were fitted using iterative nonlinear regression with parameter estimates from the turning point tests as initial values. In class 1 neither population was cyclical, except that the algae in study 12 showed a long-period seasonal cycle; in class 3 *Daphnia* cycled and algae did not. Each data set contained at least 15 sampling dates and 3 months' data after the spring decline, and almost all had more than 23 observations. Note that in studies from refs 9, 10 and 15, different classes occur in different years in the same system. NA, not applicable; NS, cycle found, not significant.

* Year in which data were collected

† Confidence limits based on the sequence of periods found in each study.

these slow seasonal changes in food supply and temperature without strong fluctuation. (2) Predator-prey cycles. *Daphnia* and phytoplankton oscillate at close to the same frequency but out of phase, in what seem to be predator-prey cycles. *Daphnia* and algae, more than most herbivore-plant systems, conform to the structure of predator-prey models in that algal individuals are small relative to *Daphnia* and are eaten whole. There is the added advantage that there is no additional basic trophic level, as in true predator-prey systems, which is only implicit in typical predator-prey models. (3) 'Single-species cycles'. *Daphnia* again oscillate, up to six times after the spring decline, but the phytoplankton appear not to oscillate even though they have a very short generation time relative to *Daphnia*. Any fluctuations in phytoplankton that do occur appear unrelated to *Daphnia* fluctuations. This pattern is similar to that observed in some laboratory populations of *Daphnia* provided with a fixed food supply, hence the title for this class.

We confirmed our subjective classification by a series of statistical analyses designed to detect cycles, estimate their period and test their statistical significance. All studies fit unambiguously into one of the three classes (Table 1). Figures 1–3 show one example from each class. Analysis of other populations might of course uncover additional classes of dynamics.

The results provide quantitative confirmation that there are distinguishable classes of population dynamics in the *Daphnia*-algal interaction, and that populations can be either cyclical or non-cyclical. The periods discovered in class 2 and 3 populations (23–47 days at average temperatures of 15–24°C) coincide closely with the range observed in cyclical laboratory populations (temperature range 14–20°C). It would have been possible to detect cycles with periods of almost 100 days in all cases and up to ~200 days in some, so the congruence with laboratory results is probably not fortuitous.

The most common behaviour was predator-prey cycles (class 2) (10 examples). There were five stable driven systems (class 1). After removal of a seasonal trend, these showed either no oscillation or one with a very long period reflecting seasonal change not captured by the simple regression (Table 1). Only two examples were of single-species cycles (class 3), although there are at least three others, for which we do not have the original data, which appear to be class 3^{34,35}.

Two important findings are that different dynamics are sometimes seen in the same lake in different years, and that different classes of dynamics are seen in the same species. The stock tank populations also showed different dynamics in the same species at the same time (Figs 1, 3).

Class 3 dynamics (in which the prey does not oscillate even though it has a very short response time and can oscillate) have not previously been demonstrated to occur in natural populations. Other systems exist in which the predator oscillates and the prey does not, or at least not on a timescale as short as that of the predator, for example, insect pests in forests³⁶ (where the pest is the predator and trees are the prey) and some disease organisms in human populations (where humans are the prey³⁷). Prey oscillations at the same frequency as those of the predator, however, are demographically impossible because of the long generation time of the prey in these situations. (In plankton, by contrast, prey generation time is measured in days, predator generation time in weeks.) The 4- and 10-yr cycles in small mammals could be the predator component of class 3 dynamics. Unfortunately, too little is known about the dynamics of the vegetation to reach a conclusion. In addition, the cycles may be internally driven by individual behaviour, and true predators of the small mammals seem to play a part in the cycles in many cases³⁸.

We cannot explain the apparent absence of oscillations in the

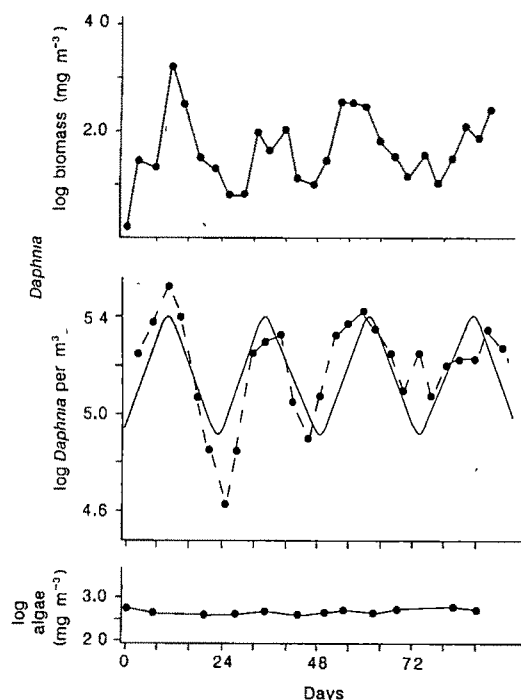


Fig. 3 Class 3 dynamics from tank experiment. log *Daphnia* abundance per m^3 and log *Daphnia* biomass ($mg\ m^{-3}$) oscillate, while log algal biomass ($mg\ m^{-3}$) is stable. *Daphnia* biomass (dry weight) was estimated using data on abundance and dry weights (derived from length-weight regressions⁴²). These experimental populations were run at the same time as those described in Fig 1.

algae when they are so clearly marked in the *Daphnia*. We believe the difference between algal and *Daphnia* dynamics is real, but two alternatives, based on the assumption that the difference is an artefact, need to be considered. First, the fluctuations in *Daphnia* density might be caused mainly by changes in age structure, with a roughly constant *Daphnia* biomass being maintained by the roughly constant algal biomass. Figure 3 shows that this is not the case in the one example of this class for which we have detailed information on the sizes of individuals; total biomass also cycles. Second, fluctuations might occur in the edible phytoplankton but would not be seen in the algal data (usually chlorophyll *a* concentration), which reflect the abundance of all algae, edible or not; this implies that the edible and non-edible fractions are negatively correlated in time. This explanation cannot be ruled out for all cases, but it appears not to be valid for Edmondson and Litt's study³⁴ in which the edible fraction was estimated. A third possibility is that the algae do indeed oscillate, but over such a small range that the oscillations are not detected.

Our demonstration that structurally similar systems can exhibit a broad range of dynamic behaviour suggests that it is valid to search for general models which seek to explain the variety of observed dynamic behaviour as resulting from quantitative differences contained within a common structure². In the particular system studied here, our initial hypothesis is that the existence of each broad dynamic pattern can be explained solely by the interaction between *Daphnia* and its food supply. We expect that whether class 1 or class 2 dynamics is observed in a particular lake will be determined by the manner in which factors such as nutrient levels and average temperature influence ecological properties (such as algal rate of increase) of the populations. We are now testing this hypothesis.

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Light-dependent antibody labelling of photoreceptors

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Monoclonal antibodies are tools widely used to analyse the structure of the nervous system. Whereas some labelling patterns are highly reproducible, others appear to vary from one preparation to the next, as we noticed in particular for some antibodies with respect to photoreceptor labelling. To establish whether some of this variability can be linked to functional criteria, we tested for light-dependence. We found two antibodies that label photoreceptor outer segments, only when the retina has been illuminated, and a third antibody that has a selective affinity for dark-adapted outer segments. The two antibodies against light-activated sites are primarily directed against the highly phosphorylated neurofilament subunit at relative molecular mass 200,000 (200K). One of them RT97, recognizes on immunoblots, in addition to neurofilaments, a light-activated epitope on a protein that resembles the photopigment rhodopsin, presumably a phosphorylation-dependent site. Antibodies like those described here may allow the study of physiological processes such as light and dark adaptation using morphological techniques.

As many physiological events in the nervous system are executed through reversible covalent modifications in molecules, it should be possible to find antibodies selective for particular states of the sites that undergo changes. We found previously that antibodies against the heavy (200K) neurofilament subunit can reveal neuronal reactions to injury; these changes were inducible over a time course of days¹. Some of the same antibodies detect modifications in mitotic cells which appear and vanish within about 1 h (ref. 2). Our studies revealed that two

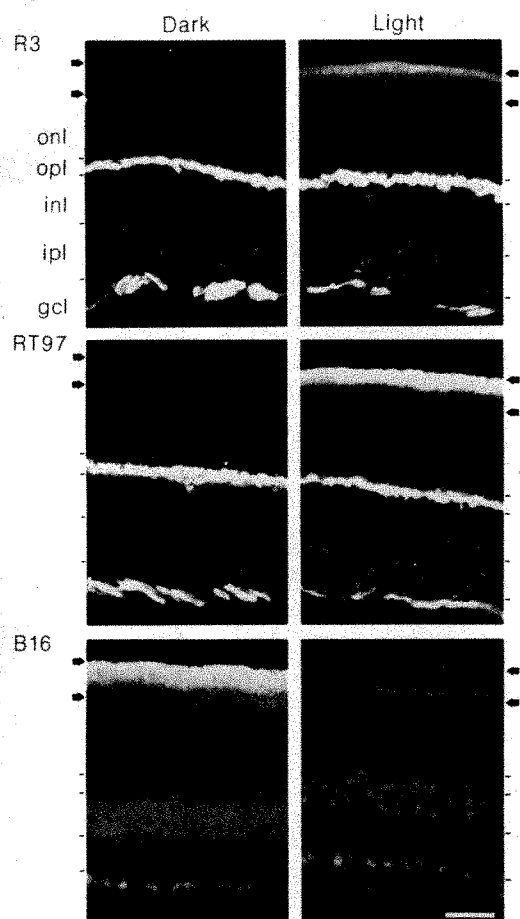


Fig. 1 Transverse sections through dark-adapted (left vertical lane) and light-adapted (right lane) retinas labelled with the antibodies R3 (ref. 4) (top row), RT97 (ref. 3) (middle) and B16 (ref. 6) (bottom). The layer of photoreceptor processes, including inner and outer segments, are indicated by the arrows on the left and right margins; other retinal layers are labelled as: onl, outer nuclear layer; opl, outer plexiform layer; inl, inner nuclear layer; ipl, inner plexiform layer; gcl, ganglion cell layer. Note that the two neurofilament antibodies RT97 and R3 label photoreceptor outer segments only in the light-adapted condition; the labelling of neurofilaments in horizontal cells and optic axons is independent of the illumination history. Antibody B16 has selective affinity for dark-adapted outer segments. Scale bar, 50 μ m.

neurofilament antibodies of the same class, and another unrelated antibody, can be used to detect changes that occur within seconds to minutes in photoreceptor outer segments in response to light and darkness.

C57BL/6J mice were anaesthetized with pentobarbital, their body temperature was maintained at 36.5 °C, and they were dark adapted for 45 min; one eye was taped shut and the other was either diffusely illuminated or was covered with a contact lens and exposed to stationary visual patterns. After ~2–8 min, the mice were quickly perfused under dim red light with 4% paraformaldehyde/0.12% picric acid; the retinas were left as whole mounts or sectioned on a cryostat and processed for indirect immunofluorescence, using fluorescein-isothiocyanate labelled secondary antibodies (Boehringer Mannheim). Of 10 different monoclonal antibodies against 200K neurofilaments that we tested, only two showed light-dependent photoreceptor labelling: the antibodies RT97 (ref. 3) and R3 (ref. 4). The third antibody described here, B16, was generated following the protocol of Matthew and Patterson⁵ in a fusion aimed at obtaining antibodies against photoreceptors⁶: a BALB/c mouse was first immunized with mutant retina lacking photoreceptors (C57BL/6J *le-rd*), then many of the responding B cells were eliminated by intraperitoneal injection of cyclophosphamide, and finally the surviving B cells were challenged *in vitro* with

retina from normal mouse (C57BL/6J), and fused with NS1 myeloma cells⁷.

Figure 1 shows sections from dark-adapted (left) and illuminated retinas (right) labelled with these three antibodies. The antibodies RT97 and R3 reveal the characteristic neurofilament distribution which is, as expected, independent of illumination levels: these are components of horizontal, ganglion and amacrine cells. The photoreceptor outer segments, however, are labelled only in the illuminated retinas and negative in the dark-adapted ones. As there are no neurofilaments in outer segments, the light-activated labelling must reflect a chance cross-reactivity. The third antibody, B16, shows the reverse pattern with respect to the outer segments: it labels them only in the dark-adapted state. In addition this antibody labels synaptic components with high affinity and all cell nuclei with low affinity, regardless of the illumination history. All three antibodies appear to label only the outer segments of rods and not of cones, as we determined by double-labelling with cone-specific peanut lectin⁸ (data not shown).

The labelling is spatially precise: if the mouse had been viewing a striped pattern, the retina is labelled in a striped fashion, as shown for RT97 in a whole mount in Fig. 2a. If the mouse was held stationary during visual stimulation the stripes are interrupted by the shadows of the blood vessels in the inner retina (Fig. 2b). To illustrate the complementarity of labelling with the two types of antibodies, we let the mice face writing in bright letters: a retinal whole mount from such a mouse labelled with RT97 shows the writing as bright on dark (Fig. 2c), and in a B16-labelled retina the writing appears dark on a bright background (Fig. 2d).

Although both neurofilament antibodies label outer segments only in the light, they seem to recognize different antigenic sites, as indicated by light-adaptation experiments: anaesthetized mice were exposed to a brightly illuminated screen in the nasal half of their visual field with the temporal half blocked off by a black mask; after 10 min the mask was quickly pulled away to allow for short periods of full-field stimulation. The mice were then rapidly perfused under dim red light, the retinas sectioned dorsally to ventrally and examined for temporo-nasal differences in photoreceptor labelling. Such light-adaptation experiments revealed traces of the RT97 site within as little as 10 s of light exposure; over the following minutes the labelling intensity increased up to a maximum and thereafter remained constant. The R3 site behaved differently: like the RT97 site it turned on rather rapidly in response to light onset and grew to a maximum; however, it then diminished with steady illumination. This is illustrated in Fig. 3 for a mouse whose nasal retina (right) was illuminated for 10 min and 45 s, and the temporal retina (left) for 45 s only: RT97 labels the 45 s exposure less brightly than the 10 min + 45 s exposure, whereas R3 shows the reverse pattern: the retina half illuminated for 45 s is brighter. Hence under the intense illumination conditions used here the R3 site appears to respond preferentially to light transients, whereas the RT97 site seems to measure the amount of illumination.

We tried to determine the relative molecular masses of the illumination-dependent antigens. C57BL/6J mice were light- or dark-adapted for 1 h; their retinas were removed under bright white or dim red light, homogenized and dissolved in cold SDS, separated on SDS/polyacrylamide gels and blotted onto nitrocellulose paper^{9,10}. So far we have found a clear light/dark difference on blots only for RT97, as shown in Fig. 4a, which compares RT97 with the monoclonal antibody RET-P1 that recognizes rhodopsin¹²; the lanes of dark-adapted retinas (D) were loaded with twice the amount of protein as the lanes of the light-adapted preparation (L) to bias against detection of light-activated antigens. RET-P1 labels both in the light- and dark-adapted preparations three broad regions which presumably correspond to rhodopsin and its aggregates; the broad distributions reflect multiple bands that probably differ in phosphorylation states. RT97 reacts practically only with the light-adapted preparation; it recognizes mainly a broad region at

Fig. 2 Whole mounts of retinas from mice exposed to stationary visual patterns for ~8 min, and reacted with RT97 (*a-c*) and B16 (*d*). The mouse of the retina in *a* had been viewing a flat panel with parallel stripes covering most of the visual field through the stimulated eye. The labelling pattern reflects a combination of tangent and spherical distortions. In a higher power view of a different retina (*b*) a stripe, composed of labelled rod outer segments, can be seen disrupted by the shadows of blood vessels from the inner retina, indicating that the mouse did not move during visual stimulation. What at this magnification appears as slight irregularities in outer segment labelling corresponds at higher magnifications to cross-striations within single outer segments: apparently, stacks of disks partially separated because of mild detergent treatment of the whole mounts. Single labelled outer segments are visible here, because they are surrounded by unlabelled ones, which seems to reflect the mode of dark adaptation of the RT97 sites; with increasing time in the dark after a strong light stimulus, increasing numbers of outer segments lose RT97 affinity in an all-or-none fashion. The mice in *c* and *d* were exposed to the same

bright writing on a dark background: RT97 recognizes the illuminated letters and B16 the dark background. Under the gentle conditions of antibody incubation used here, RT97 did not penetrate sufficiently into the tissue to label neurofilaments except around the optic disk and at the edges of the whole mounts. Scale bars, 1 mm (*a, c, d*) and 200 μ m (*b*).

33–39K and a weaker one at 67–76K, both of which line up roughly with the two lower regions of RET-P1. In other preparations a third band just above 100K, at the approximate location of rhodopsin trimers, was very weakly labelled in a light-dependent fashion. In preparations aimed at minimizing rhodopsin aggregation (Fig. 4*b*), RT97 recognized a single light-induced band at 34–40K, in addition to light-independent bands that correspond to the 200K neurofilament subunit and its presumed breakdown products¹¹. A plausible explanation is that RT97 crossreacts with light-adapted rhodopsin; if this assumption is correct, the apparently higher affinity of RT97 for rhodopsin monomers than for aggregates, compared with RET-P1, may indicate either that the RT97 site interferes with rhodopsin aggregation or that it is less accessible in aggregates.

We do not yet know what the changes at the three illumination-dependent sites may be, but a cue to the nature of two of them comes from the affinity of the antibodies for 200K neurofilaments. This neurofilament subunit is known to be highly phosphorylated, and most 200K neurofilament antibodies were shown to label neurofilaments in a phosphorylation-dependent manner^{13–15}; they presumably recognize either a short peptide

sequence containing a phosphoserine, or a change brought about in the peptide chain by nearby phosphorylation. A phosphorylation dependent labelling applies also to the two antibodies RT97 and R3 used here: their affinity for neurofilaments can be abolished by alkaline phosphatase treatment (J. Wood, personal communication); we observed the same for isolated outer segment preparations (data not shown). A major covalent modification in photobleached rhodopsin is phosphorylation at a total of nine sites, a process thought to impede its ability to participate in the phosphodiesterase activation cycle and considered a mechanism in light adaptation^{16–19}. For RT97 a crossreactivity with one of the light-induced phosphorylation sites on rhodopsin appears likely. R3 may also cross-react with rhodopsin (although so far we could not detect such a cross-reactivity on blots) or it may recognize one of several other outer segment proteins that are phosphorylated in the light²⁰. The light-adaptation experiments indicate that the R3 site probably differs from the RT97 site and that the two sites may mediate different functions. As activation of the RT97 site increases with illumination time up to a saturated level, its behaviour is consistent with a mechanism involved in the mediation of light adaptation. The R3 site,

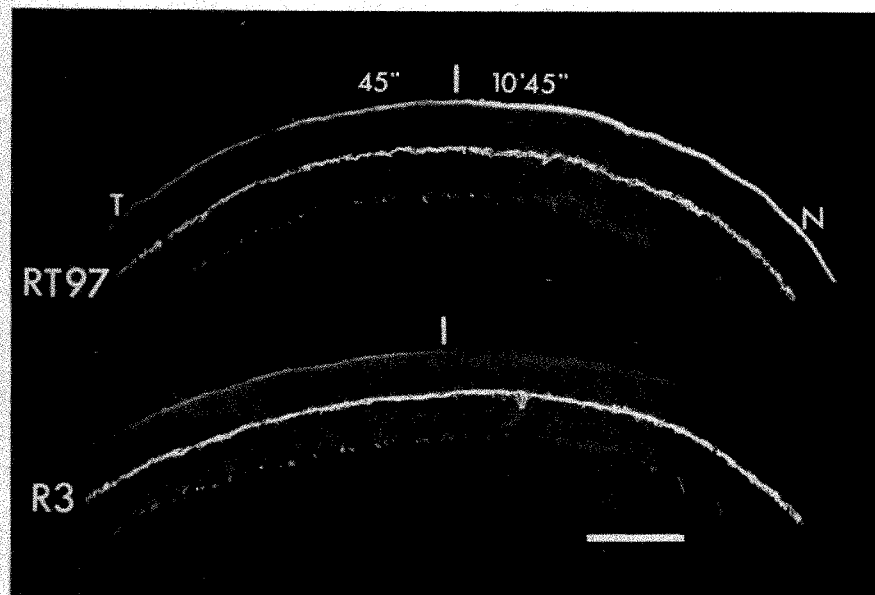


Fig. 3 Light-adaptation experiment. Transverse sections from retina exposed to bright light for 10 min in its nasal half only, and for 10 min and an additional 45 s to full-field stimulation. The border between the nasal (N) and temporal (T) halves is indicated by the white lines above the sections. Note that RT97 labels the 10 min + 45 s exposure more brightly than the 45 s exposure, whereas the R3 labelling is brighter after the shorter stimulation period. Scale bar, 200 μ m.

on the other hand, does not seem suited to mediate light adaptation, as it seems to adapt to light by itself. Alternatively, the apparent light adaptation of the R3 site may be an artefact resulting from steric hindrance of antibody binding through neighbouring additional phosphorylation sites. Finally, we have no clue to the antigen(s) recognized by antibody B16 in photoreceptors. If labelling with B16 were also related to phosphorylation events, it may be directed against one of the components in photoreceptor outer segments that are phosphorylated in the dark in a cyclic GMP-dependent manner, or it may recognize a site that is unmasked by dephosphorylation in the dark^{21,22}.

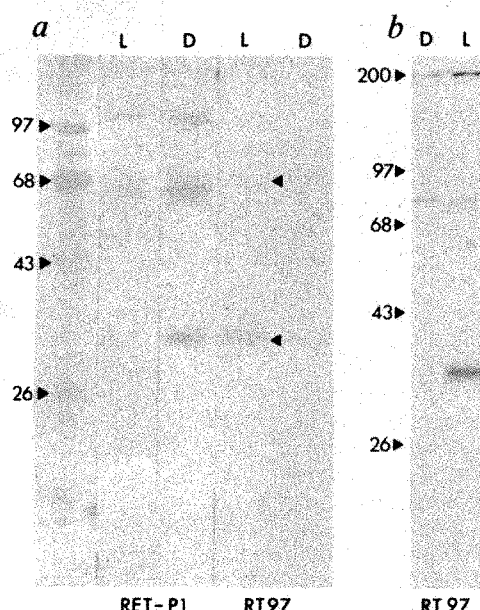


Fig. 4 *a*, Immunoblot of RT97 (ref. 3) on light- and dark-adapted retinas and comparison with RET-P1, a monoclonal antibody recognizing rhodopsin¹². Mice were either light-adapted (>100 cd m⁻²) or dark-adapted for 1 h, and killed by cervical dislocation. Retinas were quickly removed, homogenized in a buffer containing 1 mM EDTA, EGTA and phenylmethylsulphonyl fluoride, and treated with DNase (1 mg ml⁻¹). Samples were dissolved in cold sample buffer to a final concentration of 2% SDS, 5% 2-mercaptoethanol, 10% glycerol, 0.01% bromphenol blue in 0.625 M Tris HCl, pH 6.8, briefly sonicated (not boiled) and loaded onto a 12% polyacrylamide gel: 100 µg protein from dark-adapted retinas per D-lane, and 50 µg from light-adapted retinas per L-lane. Electrophoresis was performed using the buffer system of Laemmli⁹, and proteins were transferred from the gel onto nitrocellulose paper according to Towbin *et al.*¹⁰. Strips of nitrocellulose paper were either stained for protein with amido black, or blocked with 3% bovine serum albumin and processed for antibody binding using peroxidase labelled secondary antibody (Cappel) and 3,3',4,4'-biphenyl tetramine tetrahydrochloride (Sigma) as chromogen. Relative molecular mass standards were myosin (200K), phosphorylase B (97K), bovine serum albumin (68K), ovalbumin (43K) and α -chymotrypsinogen (26K). Note that RT97 recognizes two broad regions in the light-adapted lane that line up roughly with the presumed monomers and dimers of rhodopsin labelled by RET-P1. Apparent relative molecular masses of the labelled regions, as determined from this and four other gels, were 33–39K (s.d. 3) and 67–76K (s.d. 8) for RT97, in addition to a very faint band just above 100K, and 32–38K (s.d. 3), 67–78K (s.d. 7) and 103–109K (s.d. 1) for RET-P1. *b*, Light- and dark-adapted retinas blotted with RT97, prepared slightly differently to minimize rhodopsin aggregation and to demonstrate neurofilament labelling: one retina per lane was homogenized in 50 µl pH 7.4 Tris buffer containing 1 mM EDTA and 0.1 µl saturated phenylmethylsulphonyl fluoride, and incubated for 10 min with 0.017% sodium deoxycholate; the protein was precipitated by adding 6 µl of 72% trichloroacetic acid and the precipitate was resuspended in SDS sample buffer⁹ by sonication and vortexing, and treated as above. The light-independent bands are the same as seen by Calvert and Anderton¹¹ on blots of rat brain with RT97: the 200K neurofilament subunit and presumed neurofilament breakdown products.

We have shown here that monoclonal antibodies can be used to detect relatively fast physiological changes in photoreceptors. Such antibodies may allow the analysis of reversible covalent modifications at single sites, which at least for the phosphorylation sites on rhodopsin has not been possible with conventional biochemical techniques. As many physiological changes are brought about by phosphorylation events²³, the group of 200K neurofilament antibodies may provide a reservoir of detectors for functional epitopes.

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Stimulation of multipotential, erythroid and other murine haematopoietic progenitor cells by adherent cell lines in the absence of detectable multi-CSF (IL-3)

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It is well established that murine multipotential and committed erythroid progenitor cells require the presence of a glycoprotein, termed multi-CSF (multi-colony-stimulating factor, IL-3)^{1–7} for clonal proliferation and differentiation *in vitro*. The initial proliferation of these cells can also be stimulated by two other glycoproteins, granulocyte-macrophage CSF (GM-CSF) and granulocyte CSF (G-CSF), although continued proliferation and differentiation requires the subsequent presence of multi-CSF^{8,9}. Here we report the stimulation of multipotential, erythroid and other haematopoietic progenitor cells by a number of adherent cell lines including a cloned bone marrow cell line (B.Ad). The positive cell lines, as feeder layers, exhibit colony-stimulating, erythropoietin-like and burst-promoting (BPA) activities. Optimal erythropoietic stimulation by the B.Ad line requires close cell–cell contact. The cell lines also support the *in vitro* clonal growth of multipotential colony-forming cells and progenitors of six other haematopoietic lineages. The biological activities observed seem not to be mediated by known multipotential or erythroid colony-stimulating factors (multi-CSF⁷, IL-3⁵, MCGF¹⁰, HCGF³, PSF⁶, BPA²).



Fig. 1 Phase contrast photograph showing confluent cultures of B.Ad cells. Note clusters of polygonal cells heavily laden with lipid droplets. $\times 135$. The cell line was isolated from a non-adherent cell population taken from a 1-week-old liquid culture of BALB/c bone marrow cells. It was observed during weekly serial passages of the non-adherent expanding population of mast cells in growth medium (DMEM containing 20% fetal calf serum, supplemented with medium conditioned by WEHI-3B myelomonocytic leukaemic cells (WEHI-3B CM)), that there were always adherent cells growing in the cultures. On reaching confluency, cells heavily laden with lipid droplets appeared. Nine months after initiation of the cultures, non-adherent cells were removed and adherent cells collected by trypsinization and cultured alone. This cell line has been designated B.Ad (BALB/c adherent) and has now been maintained as adherent cultures for >1 yr.

B.Ad cells were derived from BALB/c bone marrow adherent cell cultures (see Fig. 1 legend). On reaching confluence, cultures of B.Ad cells displayed clusters of lipid-containing cells intermixed with fibroblastoid cells (Fig. 1). The lipid-containing cells were oil red O positive, and most cells showed weak nonspecific esterase activity, but no metachromasia, myeloperoxidase or acetylcholinesterase activity. Approximately 95% of the cells phagocytosed latex particles and had the following surface phenotype: $H-2^+$ (40–60%), Ia^- , Ig^- , Lyl^-2^- . The mean population doubling time was 76 h and the cells had a modal chromosome number of 75. Tests for tumorigenicity are in progress,

but no tumours are evident 2 months after subcutaneous inoculation of 10^6 B.Ad cells into syngeneic newborn mice.

Table 1 shows colony formation by bone marrow cells separated from the adherent layer of B.Ad cells by an intermediate layer of agar medium. Most colonies were composed of neutrophils and/or macrophages but megakaryocytes and mixed cell colonies were also observed (average of 4 per culture), although less colonies were stimulated by the B.Ad cells than in control cultures stimulated with PWM-SCM (pokeweed mitogen-stimulated murine spleen cell-conditioned medium).

The erythropoietic stimulating activity of B.Ad cells was investigated using methylcellulose cultures which allow target fetal liver cells to seed directly on top of the feeder layers. Table 2 shows the result of a typical experiment. In the absence of exogenous erythropoietin, B.Ad cells supported the growth of late erythroid (day 2 colony forming units-erythroid (CFU-E)) colonies (Table 2). Separation of fetal liver and B.Ad cells by an agar interlayer reduced the number of CFU-E colonies by 75%. Mixing experiments with either PWM-SCM or erythropoietin showed that there was no significant inhibitory or additive effect by the B.Ad cells on the growth of CFU-E colonies. When examined at day 7, small multicentric foci of poorly haemoglobinized erythroid cells were observed. In sharp contrast, in the presence of exogenously added erythropoietin, the B.Ad cells could stimulate about fourfold higher numbers of erythroid colonies which were larger and more haemoglobinized than in cultures maximally stimulated by PWM-SCM + erythropoietin. There was no significant difference observed in the number of non-erythroid colonies.

Following sequential removal, smearing and staining of all colonies in cultures of 2×10^3 fetal liver cells, pure erythroid, neutrophil, neutrophil-macrophage, neutrophil-macrophage-mast cell, neutrophil-macrophage-eosinophil and erythroid-neutrophil-macrophage-eosinophil-mast cell-megakaryocyte-blast cell colonies were observed.

The fetal liver origin of the macroscopic erythroid colonies was confirmed by the presence of a single adult haemoglobin band ($\alpha_2\beta_2$ major) characteristic of C57BL/6 mice, when C57BL/6/f/J WEHI fetal liver cells were seeded onto the B.Ad cell line.

Separation of the target cells from the adherent cells by an agar interlayer reduced the number of macroscopic erythroid colonies by $>90\%$ but did not significantly affect the number of non-erythroid colonies. In cultures containing both B.Ad cells and PWM-SCM the same high numbers of erythroid colonies developed as with B.Ad cells alone, suggesting that no inhibitors

Table 1 Morphological characterization of murine bone marrow colonies stimulated by feeder layers of B.Ad cells

Stimulus	Total colonies	% Colonies§					
		Neutrophil	Neutrophil-macrophage	Macrophage	Megakaryocyte	Erythroid (pure and mixed)	Mixed-cell
PWM-SCM*	163 $\pm$ 15‡	8.8	43.9	29.0	11.2	5.9	1.0
B.Ad†	106 $\pm$ 7	21.6	62.6	11.9	3.1	0.0	0.9

50,000 CBA/CaH bone marrow cells were seeded into 35-mm Petri dishes containing 1 ml 0.3% agar in Dulbecco's modified Eagle's medium (DMEM) supplemented with 20% fetal calf serum (FCS). Cultures were kept in a fully humidified 37 °C incubator containing 10% CO₂ in air. Cultures were scored after 7 days of incubation and were then fixed with 2.5% phosphate-buffered glutaraldehyde at room temperature overnight. Whole culture mounts were prepared and stained sequentially with cholinesterase stain, Luxol-fast-blue stain and counterstained with Meyer's haematoxylin. Colonies were typed according to criteria described previously¹⁶.

* PWM-SCM was prepared from BALB/c spleen cells as described previously¹⁷; 0.1 ml of pre-determined dilution of PWM-SCM (giving maximal stimulation) was used.

† Monolayers of B.Ad cells were prepared in 35-mm Petri dishes by splitting confluent stock cultures 1:100 and seeding cells in 1 ml medium. On reaching confluency (10–14 days), culture medium was removed and 0.5 ml 0.3% agar medium was laid on top of the cell monolayer. After gelling, 1 ml of agar medium containing bone marrow cells was overlaid and allowed to gel before incubation.

‡ Triplicate counts (means $\pm$ s.d.).

§ Results were the average of triplicate plates.

|| Mixed-cell colonies containing neutrophils, macrophages, either eosinophils or megakaryocytes or both with the apparent absence of recognizable erythroid cells.

Table 2 Increased erythroid colony formation by close contact with feeder layers of B.Ad cells

Stimulus	No. of colonies per culture					
	-Epo			+Epo		
	Day 2 CFU-E	Erythroid	Day 7 Non-erythroid	Day 2 CFU-E	Erythroid	Day 7 Non-erythroid§
PWM-SCM	324	11	63	798	17	61
MLCM*	0	0	57	828	5	32
Medium	0	0	0	870	0	0
B.Ad uncloned line	228	0	73	840	80	43
B.Ad uncloned line (A)†	54	0	59	888	6	66
B.Ad uncloned line + SCM	330	6	78	864	87	63
B.Ad uncloned line (A) + SCM	360	2	78	942	ND	ND
B.Ad‡						
Clone 2	144	0	62	828	110	37
Clone 2 (A)	72	0	54	936	8	50
Clone 3	192	0	61	756	153	35
Clone 3 (A)	36	0	56	828	5	60
Clone 4	156	19	74	648	75	37
Clone 4 (A)	12	0	65	876	6	52
Clone 6	48	0	67	456	94	44
Clone 6 (A)	24	0	53	840	4	70
Clone 7	108	0	60	528	105	44
Clone 7 (A)	72	0	55	948	7	70

Adherent layers of B.Ad cells were prepared as described in Table 1; 10,000 12-day CBA/CaH fetal liver cells were cultured in 35-mm Petri dishes in 1 ml volume of 0.78% methylcellulose (Methocel MC, premium, 4,000 cP, Dow) in Iscove's modified Dulbecco's medium containing 20% fetal calf serum and 7.5×10^{-5} M 2-mercaptoethanol. Cultures were kept in a fully humidified incubator at 37 °C with 5% CO₂ in air. CFU-E colonies were scored on day 2 and both erythroid and non-erythroid colonies were scored on day 7. Erythropoietin (Epo) was partially purified from human urine and has been shown to be free of CSF activity. 0.1 ml of predetermined dilution of erythropoietin (giving optimal stimulation of CBA fetal liver CFU-E colonies) was used. ND, not determined, or not scored.

* MLCM, mouse lung conditioned medium, was prepared as described previously¹⁸, 0.1 ml of pre-determined dilution of MLCM (giving maximal stimulation of colony growth by bone marrow cells) was used.

† A 0.5 ml, 0.3% agar-medium, interlayer was laid on top of the B.Ad feeder layers before methylcellulose medium containing target cells was plated. The presence of an interlayer neither inhibited nor enhanced colony formation in control cultures stimulated with PWM-SCM.

‡ B.Ad cells were cloned either by limit dilution method in 96-well tissue culture plates (at a density of 0.4 cells per well in 0.2 ml growth medium) or by single-cell manipulation of B.Ad cells in the log phase of growth. In the latter method, diluted cell suspensions of B.Ad cells were prepared and seeded into 15-cm Petri dishes containing pieces of coverslips. After 1 h of incubation, coverslips with single cells or doublets were located under an inverted microscope and were individually removed and transferred to 12-well tissue culture plates or 35-mm Petri dishes for clonal expansion. A total of 12 subclones was successfully derived.

§ In the absence of erythropoietin, degenerating erythroid colonies are included in the non-erythroid category. In the presence of erythropoietin degenerating erythroid colonies were rarely observed. The apparent increase in non-erythroid colony numbers after inclusion of agar interlayer reflects difficulty in distinguishing non-erythroid colonies intermixed with B.Ad cells in cultures without agar interlayer.

were present in PWM-SCM preventing colony formation by the erythroid progenitors.

Several clones of the B.Ad cell line were isolated and all exhibited biological activities similar to those associated with the parental uncloned line (Table 2).

To determine whether the observed biological activities could be attributed to multi-CSF⁷ (IL-3), we investigated whether feeder layers of B.Ad cells could support colony formation by the multi-CSF-dependent cell line, 32D cl.3 (ref. 11). It was observed that the B.Ad cells could neither support the clonal growth in semi-solid cultures (Table 3) nor the survival of 32D cells in liquid co-cultures. Furthermore, prolonged incubation of haematopoietic cells (either bone marrow or spleen) with B.Ad cells for up to 4 weeks did not result in the generation of suspension cultures of mast cells.

We next investigated whether medium conditioned by B.Ad cells or B.Ad cells and fetal liver cells contained the biological activities described previously. Even after 18-fold concentration, we could not detect erythroid, megakaryocyte, mixed cell or 32D cl.3 colony stimulating activity in the conditioned medium, although macrophage and to a lesser degree, neutrophil colonies were stimulated in agar cultures of bone marrow cells. However, when conditioned medium was added to liquid cultures for 7 days containing 3-day post-5-fluorouracil-treated bone marrow

Table 3 Failure of B.Ad cells to support the clonal growth of 32D cl.3 cells

Stimulus	No. of colonies per culture
WEHI-3B CM*	57 ± 6
WEHI-3B CM (A)†	61 ± 7
Normal saline	0 ± 0
Normal saline (A)	0 ± 0
B.Ad uncloned line	0 ± 0
B.Ad uncloned line (A)	0 ± 0
B.Ad uncloned line + WEHI-3B CM	61 ± 6
B.Ad uncloned line (A) + WEHI-3B CM	70 ± 6

32D cl.3 cells were maintained by passage in DME containing 10% FCS with 10% WEHI-3B CM as a source of growth stimulus. For assay, cells were washed three times and cultured at a density of 100 cells ml⁻¹ in DMEM containing 0.78% methylcellulose and 20% FCS. Cultures were incubated at 37 °C in an atmosphere containing 10% CO₂ in air. Colonies were scored on day 7.

* WEHI-3B CM, medium conditioned by WEHI-3B myelomonocytic leukaemic D⁻ cells.

† 0.5 ml of 0.3% agar medium was laid either in blank Petri dishes or on top of the feeder layers of B.Ad cells before 32D cells were plated.

Table 4 Comparison of B.Ad and other adherent cell lines for ability to stimulate erythroid colony formation

Stimulus	No. of colonies per culture			
	-Epo		+Epo	
	Day 2 CFU-E	Day 7 erythroid	Day 2 CFU-E	Day 7 erythroid
PWM-SCM	180	17	432	29
Medium	0	0	552	0
B.Ad clone 2	36	2	408	63
BALB/c 3T3	12	6	348	45
Swiss 3T3	24	0	396	42
RAT-2	8	0	172	25
WEHI-265	1	0	52	0
LTA-5	0	0	408	0

Adherent cells seeded at 1×10^5 cells in 35 mm Petri dishes, 24 h later 10,000 12-day CBA/CaH fetal liver cells in methylcellulose medium cultured above adherent cells as described in Table 2.

cells, generation of multipotential and progenitor cells occurred. The latter were assayed in agar cultures containing PWM-SCM and were present at three- to fourfold higher numbers in liquid cultures containing B.Ad conditioned medium than control liquid cultures containing PWM-SCM. No cells were detected in liquid cultures containing mouse lung conditioned medium as a source of GM-CSF.

The erythroid potentiating activity of the B.Ad cells was compared with five other adherent cell lines (Table 4). Three of these lines supported day 7 erythroid colony formation in the presence of erythropoietin and two were not active in this assay. A sixth cell line (NIH 3T3) did not support day-7 erythroid colony formation, although its growth was variable in the presence of the fetal calf serum required for erythroid colony growth. None of these cell lines produced detectable multi-CSF when assayed with 32D cells, but one (Swiss 3T3) stimulated a GM-CSF-, multi-CSF-dependent cell line, suggesting the presence of GM-CSF.

The present experiments suggest that the erythroid colony stimulating activity associated with the B.Ad cells was not mediated by multi-CSF and unlikely to result from GM-CSF. Evidence against the former comes from lack of stimulation of 32D cl.3 cells, and the fourfold increase in erythroid colonies (compared with PWM-SCM-stimulated controls) and presence of megakaryocyte colonies would not be expected with GM-CSF¹². The results suggest the presence of a new as yet unidentified factor having the biological effects observed. The ability of B.Ad conditioned media to stimulate haematopoietic cell generation in liquid cultures of 5-fluorouracil-treated bone marrow cells may provide a convenient method to assay this factor. A similar conclusion has been made recently using conditioned media from a number of uncloned bone marrow cell lines, or a human bladder carcinoma cell line^{14,15}.

Another possible explanation for the unexpected range of colony stimulating activities expressed by the B.Ad cells could be via stimulation of accessory cells present in the fetal liver target cell population. However, identical results have been obtained using uncloned and cloned B.Ad cells with purified populations¹³ of fetal liver progenitor cells (data not shown) and no appropriate activity was detected in medium conditioned by mixtures of B.Ad and fetal liver cells.

The present experiments also suggest that there is an additional population of erythroid progenitor cells present in fetal liver that cannot be stimulated by multi-CSF and that the stimulation is more effective if target cells and adherent cells are in close contact.

The ability of several other cell lines to stimulate day 7 erythroid colony formation in the apparent absence of multi-CSF requires further investigation. The fact that several cell lines were inactive suggests that the erythroid effect is not caused by improvement of culture conditions by some general metabolic

effect. However, the positive cell lines should provide a convenient source of conditioned media to aid identification of the proposed new factor(s) producing the reported biological effects.

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Nonrandom loss of chromosome 15 in Syrian hamster tumours induced by v-Ha-ras plus v-myc oncogenes

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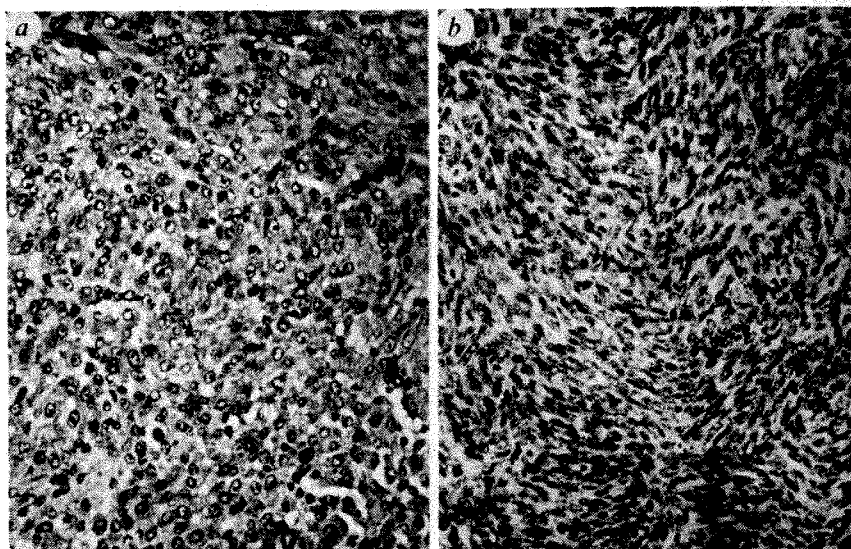
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Nonrandom chromosome rearrangements, observed in a variety of human and animal tumours^{1,2}, are associated in some cases with enhanced expression or deregulation of cellular oncogenes^{3,4}. Recently, it was shown that normal, diploid rodent cells are neoplastically transformed following transfection with two cooperating oncogenes, for example *myc* plus *ras*⁵⁻⁷. However, the number of steps necessary to convert a normal cell into a malignant cell is unknown. If activation of two oncogenes is sufficient for tumorigenicity, tumours derived from diploid cells transformed by the transfected oncogenes may remain diploid or have only random chromosome alterations. We have performed cytogenetic analyses of tumours formed after transfection of Syrian hamster embryo cells with either v-Ha-ras plus v-myc DNAs or polyoma DNA alone. Whereas polyoma-induced, tumour-derived cells were diploid, tumours induced by v-Ha-ras plus v-myc oncogenes were monoclonal and had a nonrandom chromosome change, monosomy of chromosome 15. Thus, an additional change, loss of chromosome 15, is required for or is advantageous for tumorigenicity induced by v-Ha-ras plus v-myc oncogenes. These results suggest that the neoplastic progression of normal, diploid cells requires more than two steps under certain conditions.

Early passage Syrian hamster embryo cells^{7,8} were transfected by the calcium phosphate precipitate technique^{7,9}. Cells treated with v-Ha-ras or v-myc DNA alone (see Fig. 1 legend) were repeatedly non-tumorigenic in nude mice, whereas cells co-transfected with v-Ha-ras and v-myc DNAs and injected 3 days later into nude mice formed tumours at 45-100% of the sites

Fig. 1 Histology of *v-Ha-ras* plus *v-myc*-induced tumour (2) diagnosed as pleomorphic sarcoma or fibrosarcoma with marked pleomorphism (a) and polyoma-induced tumour (4) diagnosed as a well-differentiated fibrosarcoma (b). Haematoxylin/eosin staining. ($\times 100$).

Methods. Tertiary cultures were established from 13-day-old Syrian hamster embryos as described previously⁸. Pools of primary cultures derived from littermates were cryopreserved in liquid nitrogen and secondary cultures were initiated from the frozen stocks. All experiments were performed with tertiary cultures. Plasmids [H-1 (ref. 26), pSVmyc⁵ (ref. 5) and PY1 (ref. 10)] containing genomic clones of Harvey murine sarcoma virus (*v-Ha-ras*), MC-29 viral DNA (*v-myc*) or polyoma virus DNA, respectively, were added to recipient cells by the calcium phosphate precipitate technique^{7,9}. Cells (3×10^5 per dish) were plated into 10 dishes overnight and then transfected as described with 1 μ g plasmid DNA per dish and 5 μ g of carrier calf thymus DNA. Three days after treatment, cells from each group were pooled and injected into nude mice or syngeneic newborn (3-day-old) Syrian hamsters at a dose of $4-6 \times 10^6$ cells per site and monitored for the appearance of subcutaneous tumours. No tumour formed in animals inoculated with cells treated with calf thymus DNA alone⁷. In contrast, cells treated with *v-Ha-ras/v-myc* or polyoma DNA formed tumours generally after short latency period (3-7 weeks). Tumours which formed were removed and fixed and histological sections prepared. Tumours also were minced into small (1 mm³) fragments, digested with 0.1% trypsin for 15 min, and re-established in culture.



injected, generally with a latency of 3-5 weeks⁷. These tumours, which were diagnosed as pleomorphic sarcomas or fibrosarcomas (Fig. 1a), grew progressively, reaching more than 3 cm in diameter in 2-3 weeks after detection, and were transplantable in nude mice. Cytoplasmic RNA dot-blot analysis indicated that the cells derived from the tumours expressed both *v-Ha-ras* and *v-myc* oncogenes (Fig. 2). Syrian hamster embryo cells treated with the plasmid PY1 containing the three polyoma genes¹⁰ (large-T, middle-T and small-T) were also tumorigenic, forming tumours at 21 of 22 sites injected⁷. The tumours from polyoma-treated cells had essentially the same latency period (4-7 weeks) in nude mice as the *v-Ha-ras* plus *v-myc*-induced tumours, but grew at a slightly slower rate, requiring 4-6 weeks to reach 2-3 cm in diameter; these tumours were diagnosed as well-differentiated fibrosarcomas (Fig. 1b). Tumour-derived cells expressed polyoma sequences (Fig. 2) and were readily transplantable into nude mice. Both polyoma-treated and *v-Ha-ras* plus *v-myc*-treated Syrian hamster embryo cells also formed tumours when injected into syngeneic newborn hamsters.

Q-banded karyotypic analyses^{11,12} of cells derived from six *v-Ha-ras* plus *v-myc*-induced tumours and five polyoma-induced tumours were performed at the first passage (Table 1). The modal karyotypes of all five polyoma-induced tumours (four isolated from nude mice and one from a newborn hamster) were normal diploid (Table 1, Fig. 3a), although 27-40% of the metaphase spreads had abnormal karyotypes, consisting of structural or numerical changes in four out of five of the tumours. However, no consistent change was observed in metaphases from the same tumour and no common changes were observed among tumours. Defendi and Lehman have reported that tumour-derived Syrian hamster cells transformed by polyoma virus apparently remain diploid¹³, but that random chromosome changes in these cells occur with time. Others have reported nonrandom chromosome changes in polyoma virus-transformed Syrian hamster cells several months after infection¹⁴.

Although all the karyotypes of the parental Syrian hamster embryo cells examined were normal, diploid (Table 1), a mixture of XX (61%) and XY (39%) cells were present as the cultures were established from pools of cells obtained from littermates of both sexes. Therefore, sex chromosomes and marker chromosomes were used as markers to determine whether the tumours were monoclonal or polyclonal in nature. Three of five of the polyoma induced tumours had cells with both XX and XY karyotypes (Table 1), indicating that the tumours were polyclonal in origin.

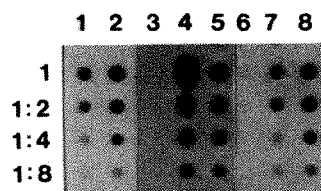


Fig. 2 RNA dot-blot analysis of polyoma and *v-Ha-ras* plus *v-myc* tumour-derived cell lines. Cytoplasmic extracts prepared from polyoma-1 (lane 1) and polyoma-2 (lane 2) tumour-derived cells were dotted onto a nitrocellulose filter and hybridized with ³²P-labelled polyoma DNA insert from clone PY1 (ref. 10). Cytoplasmic extracts prepared from Syrian hamster embryo cells (lanes 3, 6), *ras/myc*-1 (lanes 4, 7) and *ras/myc*-2 tumour cells (lanes 5, 8) were dotted onto nitrocellulose filters and hybridized with either the ³²P-labelled *v-Ha-ras* insert from clone BS9 (ref. 27) (lanes 3-5) or the ³²P-labelled *v-myc* insert from clone pBRmyc²⁸ (lanes 6-8).

Methods. Extracts from 2×10^6 tumour-derived cells or normal cells prepared by the method of White and Bancroft²⁹, and four aliquots representing $0.25-2.0 \times 10^5$ cell equivalents were applied to a nitrocellulose sheet using a Hybri-Dot Manifold (BRL). The filters were heated at 80 °C *in vacuo* for 2 h and hybridized to DNA which had been ³²P-labelled by nick-translation ($1-2 \times 10^8$ c.p.m. μ g⁻¹). Hybridizations were performed in 1 $\times$ Denhardt's solution (0.2% each of bovine serum albumin, Ficoll and polyvinylpyrrolidone), 5 $\times$ SSC (1 $\times$ SSC is 0.15 M sodium chloride, 0.015 M sodium citrate), 50% formamide, 50 mM potassium phosphate (pH 6.5), 0.25% SDS, 200 μ g ml⁻¹ salmon sperm DNA, and 10^6 c.p.m. ml⁻¹ for 48 h at 42 °C. Filters were washed in $0.1 \times$ SSC, 0.1% SDS at 50 °C and autoradiographed.

In contrast to the polyoma-induced tumour cells, the cells from the *v-Ha-ras* plus *v-myc*-induced tumours were karyotypically abnormal (Table 1). All of the cells from the *ras/myc*-1 tumour were of XX origin and had two common chromosomal abnormalities in all of the metaphase spreads: monosomy 15 and a translocation (X') between an X chromosome (at the breakpoint Xpa7) and an unidentified chromosome. However, detailed banding analysis revealed that this unidentified chromosome segment is quite distinct from chromosome 15. Half of the cells had two X' chromosomes (the modal karyotype of the tumour cells was 44, XX'X', -15) (Fig. 3b). These results suggest that the *ras/myc*-1 tumour is monoclonal and X' and -15 are early karyotypic changes. The vast majority of *ras/myc*-2 tumour

Table 1 Cytogenetic findings in Syrian hamster tumours induced by v-Ha-ras plus v-myc or polyoma oncogenes

Normal or tumour cells	Oncogene transfected	Latency (weeks)	Modal chromosome no.	Modal karyotype	Common chromosome changes	Sex chromosome distribution (XX:XO:XY)*	Remarks
SHE (Normal)	None	>25	44	44,XX	-	61:0:39	Parental cells. No cells with abnormal karyotype observed
PY1	Polyoma	6	44	44,XX	-	49:0:1	40% of the cells have abnormal karyotype
PY2		4	44	44,XX	-	29:0:21	No cells with an abnormal karyotype
PY3		7	44	44,XY	-	0:0:50	27% of the cells have abnormal karyotype
PY4		5	44	44,XY	-	8:0:42	30% of the cells have abnormal karyotype
PY5†		4	44	44,XX	-	0:0:50	36% of the cells have abnormal karyotype
<i>ras/myc-1</i>	v-Ha-ras + v-myc	5	44	44,XX'X', -15	-15,X'	50:0:0	No cells with a normal karyotype
<i>ras/myc-2</i>		5	42	42,X,-X,-15	-15	11:37:2	Cells with XY chromosome were normal§
<i>ras/myc-3‡</i>		21	42	None of the cells had the same karyotype	-10,-11,-15	0:0:50	No cells with normal karyotype
<i>ras/myc-4†</i>		4	45	46,Y,+1,+14,-15,+19,t(Xq;9p), 6q+	-15,t(Xq;9p)	0:0:50	Two cells were 44,XY; tumour formed in newborn male hamster§
<i>ras/myc-5‡</i>		7	43	43,X,+3,-15	-X or -Y, +3,-15	0:50:0	90% of the cells had double minute chromosomes. No cells with normal karyotype
<i>ras/myc-6‡</i>		4	43	43,XY,-15	-15	0:0:50	No cells with a normal karyotype

Tumours were induced following transfection with the indicated plasmids, as described in Fig. 1 legend. Tumour-derived cell lines were established in IBR-modified Dulbecco's Eagle's reinforced medium (Gibco) supplemented with 3.7 g l⁻¹ sodium bicarbonate, 20% (v/v) HyClone fetal bovine serum (Sterile Inc., Logan, Utah), 100 U ml⁻¹ penicillin and 100 µg ml⁻¹ streptomycin (Gibco). Chromosome slides were prepared by the method described previously³⁰ from the tumour-derived cell cultures at first passage. For chromosome counts, the Giemsa-stained slides were used. For banding analyses, 50 Q-banded metaphase spreads were photographed and analysed. Common chromosome changes are abnormalities observed in all the cells with an abnormal karyotype.

* In *ras/myc-1*, half of the cells (25/50) contained XX' sex chromosomes; the X' was duplicated in the remaining 25 cells, that is, XX'X'. Thus, all 50 cells were considered to be of XX origin. In *ras/myc-2*, the 11 cells with an indicated XX constitution included 10 with XX and 1 with XXX. In *ras/myc-4*, all of the 50 cells contained a Y chromosome and 48 of 50 cells had an abnormal X chromosome, that is, del(Xq); the deleted segment was probably translocated to the short arm of chromosome 9. Thus, all the cells were considered to be of XY origin.

† Tumours formed in syngeneic newborn Syrian hamsters.

‡ Karyotypic analyses of cells obtained directly from the tumours and/or from tumour-derived cell cultures at first passage. Both methods showed similar karyotypic results; the data from the direct preparation are shown.

§ A small percentage (4%) of the *ras/myc-2* and *ras/myc-4* tumours showed a normal diploid karyotype (44,XY); these cells may have been of host origin (in the case of *ras/myc-4*) or may represent a rare subpopulation of normal diploid tumour cells, or non-tumour cells that exist in the tumour.

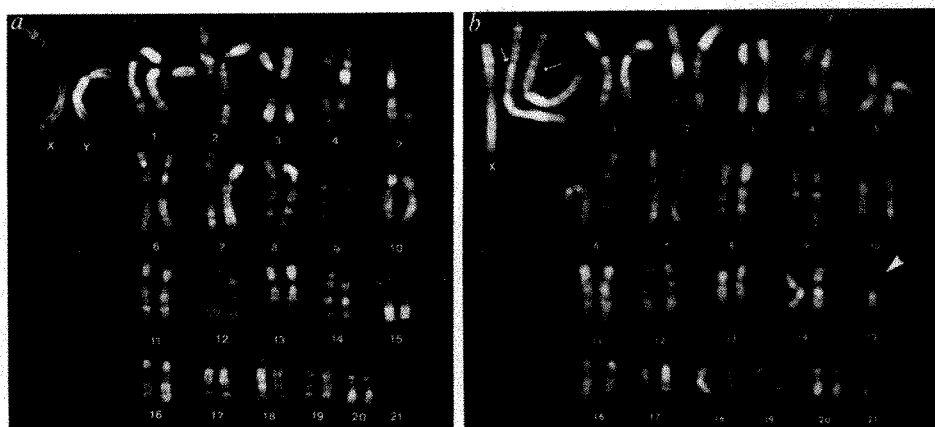
cells had also lost a chromosome 15; out of 50 cells examined, monosomy of chromosome 15 was the sole karyotypic change in 11 cells and 37 cells were monosomic for both the X chromosome and chromosome 15. Two of the cells had a normal karyotype (44,XY) (Table 1). Because 96% of the cells were monosomic for chromosome 15 and in some cases this was the sole karyotypic change, loss of chromosome 15 was probably the first karyotypic change in the transformed cells, with loss of the X chromosome an additional change, which occurred later.

The *ras/myc-3* tumour had a longer latency period than the other tumours and the karyotypes of the cells were more diverse than those of the other tumour-derived lines. No modal karyotype was evident; however, all cells displayed karyotypic alterations and losses of chromosomes 10, 11 and 15 were common changes in all of the metaphase spreads examined (Table 1). The *ras/myc-3* tumour was also examined after a culture period

of only 2 hours to obtain mitotic cells directly from the tumour; the karyotypes of these cells had the same common abnormalities as the cells at first passage. Cells from the *ras/myc-4* tumour, which formed in a male, newborn hamster, were monosomic for chromosome 15 and had a translocation between the X chromosome and chromosome 9, that is, t(X;9) (qa5-7; pa4) (Table 1). Two of the 50 cells examined were normal 44,XY but these may have originated from the male host. All the cells from the *ras/myc-5* tumour showed the absence of an X or Y chromosome and monosomy 15, together with an additional chromosome 3. No karyotypically normal cells were observed. This tumour was also examined when it was still relatively small (~5 mm in diameter) and all the cells analysed directly from the tumour showed monosomy of chromosome 15. All *ras/myc-6* tumour cells had lost a chromosome 15 and no cells with a normal karyotype were observed.

The results presented here indicate that, in contrast to the

Fig. 3 *a*, Q-banded normal, diploid karyotype of a polyoma-induced tumour (2) at first passage. *b*, Q-banded karyotype of a cell with 44,XX,X⁻15 karyotype in *ras/myc*-1 tumour at first passage. The two arrows indicate two X⁻ chromosomes, and the arrowhead indicates a missing chromosome 15. The translocation (X¹) was between the X chromosome (at the breakpoint Xpa7) and an unidentified chromosome. In this cell, two X¹ chromosomes are seen. Chromosome slides were prepared by the method described previously³⁰. The cells were banded with the quinacrine fluorescence dye for karyotypic analysis¹¹. The nomenclature of Syrian hamster chromosomes proposed by Li *et al.*¹² was used.



findings with the polyoma-induced tumours, the v-Ha-*ras* plus v-*myc*-induced tumours were karyotypically abnormal and the cells from all six tumours had a nonrandom chromosome change, that is, monosomy of chromosome 15. Furthermore, the presence of a single sex chromosome and marker chromosomes in the v-Ha-*ras* plus v-*myc*-induced tumours indicates that most of the cells in the tumours are monoclonal in nature. The monoclonality of the tumours may result from a low efficiency of transfection. However, the cells used in the experiments efficiently express pSV2neo DNA following transfection⁷ and the majority of the polyoma-induced tumours were polyclonal. These findings, although not conclusive, suggest that an additional, rare change is needed for these tumours to develop. As we have identified the nonrandom loss of chromosome 15 as the possible additional change, the monoclonal nature of the *ras/myc* tumours is consistent with this hypothesis.

We cannot determine whether the loss of one chromosome 15 is an early event necessary for the genesis of tumours induced by v-Ha-*ras* plus v-*myc* or whether this change represents a cellular progression that occurs with a high frequency and is strongly selected. The latency periods of the tumours were unrelated to the nonrandom chromosome change, but the diploid polyoma-induced tumours grew more slowly and were more differentiated in appearance than the aneuploid *ras/myc* tumours. However, the fact that the polyoma-induced tumours did not show monosomy of chromosome 15 must indicate either that this change does not result in a selective advantage for the polyoma-transformed cells or that transfection with v-Ha-*ras* and v-*myc* DNAs induces this change more frequently than treatment with polyoma DNA. It is interesting to consider whether the loss of chromosome 15 is associated with any particular stage such as immortalization¹⁵ or tumorigenicity in the neoplastic progression of the Syrian hamster embryo cells. Karyotypic analyses of carcinogen-induced and spontaneously immortalized Syrian hamster embryo have not revealed any association with monosomy 15; in fact, a nonrandom gain of another chromosome (11) was observed¹⁶. However, transfection of immortalized cell lines with v-Ha-*ras* alone results in tumorigenic transformation⁷ and with some cell lines, a nonrandom loss of chromosome 15 was observed (unpublished data). Thus, the existing data indicate that the loss of chromosome 15 is closely associated with a later stage in the neoplastic progression of these cells.

Nonrandom chromosome loss has been observed in a variety of human and animal tumours and transformed cells^{1,2,17-19}. Pathak *et al.*²⁰ observed that 15 of 18 virus (simian adenovirus-7, chicken embryo lethal-orphan, herpes simplex and simian papova viruses)-transformed Syrian hamster cell lines were monosomic for chromosome 15. This result is similar to our findings with *ras/myc*-induced tumours.

We interpret our results to indicate that the loss of a specific chromosome results either in the loss of a cellular gene which effects a phenotypic change necessary or advantageous for neoplastic development or in the deletion of *trans*-acting cellular

sequences³ which regulate the expression of either the v-Ha-*ras* or v-*myc* genes. This additional change either occurred spontaneously in these cells or may have been induced by transfection with v-Ha-*ras* plus v-*myc* DNAs. Several authors have also proposed that the dosage of cellular factors regulates the expression of tumorigenicity in viral and chemically transformed cells²¹⁻²³. Chromosome loss from hybrids between normal and malignant cells^{24,25} results in the expression of tumorigenicity. In contrast to cell hybridization studies which suggest that tumorigenicity is a recessive phenotype^{24,25}, DNA transfection studies have indicated that tumorigenesis is due to dominantly acting genetic elements²⁶. Our findings that chromosome loss can occur after DNA transfection and tumour formation may help to resolve these seemingly disparate conclusions. The oncogene-transfection system may be useful for understanding chromosomal control in carcinogenesis and the role of cellular factors in the regulation of the expression of oncogenes and tumorigenicity.

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EGF receptor-associated DNA-nicking activity is due to a M_r -100,000 dissociable protein

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The receptor for epidermal growth factor (EGF) is a single-chain transmembrane polypeptide of relative molecular mass (M_r) 170,000 (170K) which has been implicated in the regulation of both normal and abnormal cell proliferation¹. It has an externally facing EGF-binding domain and a cytoplasmically facing tyrosine-specific protein kinase site. Although the receptor has been well characterized²⁻⁴, the mechanism by which it transmits the growth stimulatory signal from the plasma membrane to the nucleus is unclear. EGF binding to cells has been shown to enhance topoisomerase activity within the cells⁵. Topoisomerases catalyse the interconversion of topological isomers of DNA and thus may influence replication and transcription⁶. Mroczkowski *et al.*⁷ reported that purified EGF receptors of both human and murine origin can nick supercoiled double-stranded (ds) DNA in an ATP-dependent fashion, an activity related to those of topoisomerases⁶. Another related tyrosine kinase, pp60^{src}, has also been reported to have a similar DNA-nicking activity⁷. We have now characterized the EGF receptor-associated DNA-nicking activity by sucrose gradient centrifugation. Our results, presented here, indicate that the DNA-nicking activity is not intrinsic to the EGF receptor, but is found in a distinct molecular species.

The EGF receptor was solubilized and purified from plasma membranes of human A431 epidermoid carcinoma cells⁷⁻¹⁰. Receptor at various stages of purification (involving chromatography on EGF-AffiGel, wheat-germ lectin(WGA)-Sephacrose, and DNA-agarose) was tested for its ability to convert pBR322 DNA from form I to form II. Figure 1 shows the results obtained with equivalent amounts of receptor (measured by EGF-binding activity) at different stages of purification. As found previously, the DNA-nicking activity was ATP-dependent (Fig. 1a) and sensitive to KCl (Fig. 1b) and NaCl (not shown).

Form I DNA was converted to form II with no evidence of intermediate topoisomers, suggesting that the observed activity is an ATP-dependent nicking of supercoils rather than a classical topoisomerase activity. Further purification of the receptor on WGA-Sephacrose revealed that while over 80% of the EGF-binding activity was adsorbed to the column, most of the DNA-nicking activity was recovered in the unadsorbed fraction (not shown). The material eluted from the WGA-Sephacrose had markedly less DNA-nicking activity (per EGF-binding unit) than did the preparation loaded onto the column (Fig. 1). Nevertheless, the remaining DNA-nicking activity was still ATP-dependent and KCl-sensitive (Fig. 1b). These results demonstrate that the DNA-nicking activity found in the EGF-AffiGel-purified receptor preparation does not co-purify with the EGF receptor through the WGA-Sephacrose step.

To confirm that EGF-binding and DNA-nicking activities are distinct, EGF-AffiGel-purified receptor was sedimented on a 5–20% sucrose gradient. Most (~80%) of both the EGF-binding activity and tyrosine autokinase activity co-sedimented at 7.7S (Fig. 2), with a minor peak of EGF-binding activity at 12S as a result of receptor dimerization, in agreement with our previous findings¹⁰. No DNA-nicking activity was seen at either 7.7S or 12S; a single peak of ATP-dependent DNA-nicking activity was seen at ~5.5S (M_r ~100,000), clearly separated from the EGF-binding and tyrosine kinase activities. Receptor further purified through WGA-Sephacrose and DNA-agarose gave identical results (not shown).

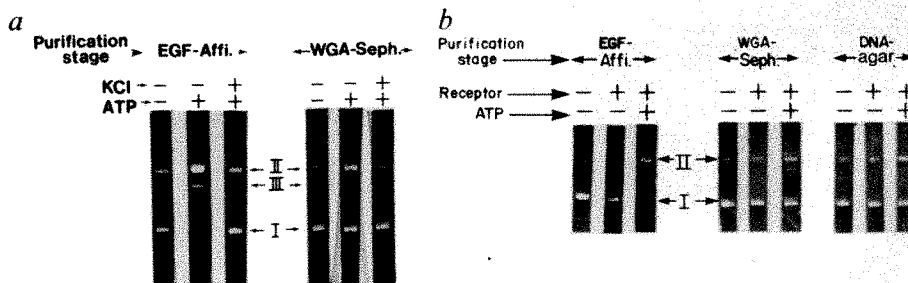
The results described above suggest that DNA-nicking activity is not an intrinsic activity of the 170K EGF receptor polypeptide. It is possible, however, that the DNA-nicking activity is the novel activity of a proteolytic cleavage product of the receptor. Trypsin treatment might thus be expected to enhance the DNA-nicking activity of receptor preparations; however no such increase was found (data not shown). Thus, evidence now available points to the conclusion that the observed DNA-nicking activity is due to an associated enzyme not related to the EGF receptor.

Note that the ratio of DNA-nicking protein to EGF-receptor protein is extremely small in our purified preparations. This is evident in the lack of detectable 100K protein (or proteins of lower M_r) in silver-stained gels (data not shown), and also in the microgram quantities of receptor protein needed for detection of DNA-nicking activity (Fig. 1).

Is the association between tyrosine kinases and ATP-dependent DNA-nicking proteins purely fortuitous? Topoisomerases are known to be bound to the 5' end of the nicked DNA through

Fig. 1 Separation of DNA-nicking activity and EGF-binding activity during WGA-Sephacrose chromatography. Supercoiled pBR322 DNA (0.2 µg) was incubated with an equivalent amount (0.5 pmol) of EGF receptor at the indicated stages of purification (amount of receptor was deduced by Scatchard analysis of ¹²⁵I-EGF-binding data). *a*, DNA-nicking activity in the presence and absence of receptor and ATP. *b*, Receptor-mediated DNA-nicking activity in the presence and absence of ATP and KCl. EGF-Affi., EGF-AffiGel; WGA-Seph., WGA-Sephacrose; DNA-agar., DNA-agarose.

Methods. Agarose gel electrophoresis was performed on 0.8% agarose gels in 40 mM Tris-acetate pH 7.9, 20 mM sodium acetate at room temperature for 18 h at 100 mA. The gels were stained with ethidium bromide (1 µg ml⁻¹), de-stained in water, and photographed under ultraviolet illumination. EGF receptor was solubilized from A431 plasma membranes and subjected to EGF-AffiGel chromatography^{3,8,9}. The EGF-AffiGel-purified receptor was subjected to further purification WGA-Sephacrose (Vector Laboratories) and ds DNA-agarose (Sigma)⁷. EGF-binding activity of the receptor preparation was determined as described elsewhere⁹. The amount of receptor present was deduced by Scatchard analysis of the ¹²⁵I-EGF-binding data, assuming a 1:1 complex of EGF and receptor. The conversion of supercoiled (form I DNA) to nicked DNA (form II DNA) was carried out as described⁷ in 20 µl of 20 mM HEPES pH 7.4, 5% glycerol, 0.1% Triton X-100, 5 mM MgCl₂ containing 0.5 pmol of receptor (or no receptor), 5 mM ATP (or not ATP), 0.15 M KCl (or no KCl). After a 30-min incubation at 20 °C the reaction was stopped by adding 5 µl of 10% SDS. Alternatively, the DNA was precipitated by adding 3 µl of 3 M sodium acetate, and 70 µl of absolute ethanol at -20 °C. The products were analysed by agarose gel electrophoresis.



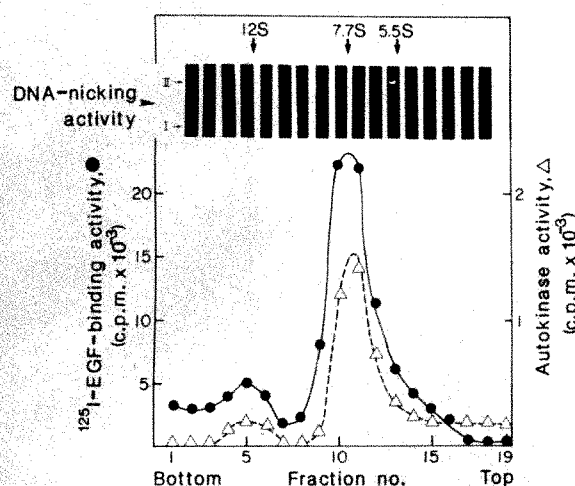


Fig. 2 Separation of the DNA-nicking activity and EGF-binding activity by sedimentation analysis. Sedimentation rates and M_s were estimated as described elsewhere^{10,14}. Approximately 10 pmol of EGF-Affigel-purified receptor (in 100 μ l of 20 mM HEPES pH 7.4, 0.2% Triton X-100 and 3% glycerol) was centrifuged at 4°C for 9.5 h at 45,000 r.p.m. in a SW 50.1 swinging bucket rotor, through a 5–20% sucrose gradient (5 ml) in 20 mM HEPES pH 7.4, 0.2% Triton X-100 (ref. 10). The standard proteins (applied to separate tubes) were: bovine serum albumin (4.6S), human IgG (7S), and horse catalase (11S). At the end of the run, the tubes were punctured at the bottom and fractions were collected. Assays for standard proteins, EGF binding and autophosphorylation have been described elsewhere¹⁰. The EGF-binding values plotted (●) are specific binding data with 150 nM ¹²⁵I-EGF (150,000 c.p.m. ng⁻¹) which were obtained after subtraction of nonspecifically bound radioactivity (~4,000 c.p.m.). Receptor autophosphorylation (Δ) was quantified, after SDS-polyacrylamide gel electrophoresis⁸ of the gradient fractions reacted with 5 μ M [γ -³²P]ATP (100 c.p.m. fmol⁻¹), by counting gel slices containing the 150K/170K receptor band¹⁰. For assay of DNA-nicking activity, 100 μ l of each fraction was incubated with 10 μ l of a solution containing 50 mM MgCl₂, 40 mM ATP and 0.2 μ g of supercoiled pBR322 DNA. After incubation at 20°C for 30 min, the DNA was precipitated by adding 12 μ l of 3 M sodium acetate and 360 μ l of absolute ethanol at -20°C. The precipitate was subjected to agarose gel electrophoresis as described in Fig. 1 legend.

a phosphotyrosine linkage^{11,12}. The phosphorylation of this critical tyrosine by pp60^{src} (like the EGF receptor, a tyrosine-specific protein kinase) causes a drastic loss of topoisomerase activity¹³. Thus, it is probable that topoisomerase-related proteins associate with tyrosine kinases such as EGF receptor in an enzyme-substrate sense.

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Mitochondrial class II introns encode proteins related to the reverse transcriptases of retroviruses

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Organelle introns share several distinctive features that set them apart from their counterparts in nuclear-encoded pre-messenger RNAs (reviewed in ref. 1): (1) their termini do not obey the GU...AG rule; (2) the introns are 'structured' (members of the same family or 'class' can theoretically adopt very similar RNA secondary conformations^{1–3} and several of the postulated pairings have been confirmed by studies of splicing mutants and their revertants (see, for example, ref. 4); (3) many introns from both classes contain long open reading frames^{4–10}. We report here that the proteins potentially encoded by four class II introns are related to several RNA-dependent polymerases of viral and transposable element origins.

Four mitochondrial members of class II contain long open reading frames: introns a1 and a2 in the cytochrome oxidase subunit I gene of the yeast *Saccharomyces cerevisiae*⁵; an intron in the corresponding gene of the filamentous fungus *Podospora anserina*¹¹; and the single intron in the apocytochrome *b* gene of *Schizosaccharomyces pombe*¹². The sequences of these introns are sufficiently similar to be aligned over most of their length. Recently, the protein potentially encoded by a mitochondrial plasmid of the Mauriceville-1c strain of *Neurospora crassa*¹³, another filamentous fungus, was shown to constitute the fifth known member of this family¹². The relationship is rather distant, as alignment of the plasmid-encoded protein with the four intronic ones is only possible over short amino-acid stretches (blocks I–VII in Fig. 1), all located in a section that does not span more than one-third of the estimated length of the molecules.

The possibility of the Mauriceville plasmid being related to transposable elements was suggested by Nargang *et al.*¹³. To test this hypothesis, we compared the seven amino-acid stretches conserved in the plasmid putative protein product and its intronic relatives with those characteristic of a family of RNA-dependent polymerases, which includes the products of the *pol* genes of retroviruses and a *copia*-like element as well as the related proteins encoded by cauliflower mosaic and hepatitis B viruses^{14–16} (segments III, IV and V in Fig. 1 correspond to segments I, II and III, respectively, of ref. 15).

As shown in Fig. 1, each of the mitochondrial conserved segments has a counterpart in viral sequences. Moreover, not only is the order of conserved blocks the same in the two sets, but distances (in amino acids) from one block to the next are rather similar, with two exceptions: the four intronic proteins (but not that encoded by the Mauriceville plasmid) have large insertions of poorly conserved sequence between segments IV and V; the Mauriceville plasmid sequence (and also that of the hepatitis B viral protein) possesses a large insertion between segments III and IV. Note also that 9 of the 10 residues found by Toh *et al.*¹⁴ to be invariant in the set of RNA-dependent polymerases they compared could each be identified in at least 4 of the 5 mitochondrial sequences.

The section of the *pol* gene that has a counterpart in the reading frames of class II introns is thought to be responsible for the polymerase activity (see ref. 14). Two lines of evidence have already indicated the presence of a reverse transcription

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Fig. 1 Alignment of similar segments in mitochondrial intron- and plasmid-encoded proteins and RNA-dependent polymerases. Amino-acid sequences were deduced from nucleotide sequences. Mitochondrial sequences are those of the proteins potentially encoded by introns S.c.a1 and S.c.a2 of *S. cerevisiae*⁵, P.a.a1 of *P. anserina*¹¹ and S.p.b1 from *S. pombe*¹², together with that of the putative protein product of a *N. crassa* plasmid¹³ (N.c.mau.). Retroviral sequences are those of the *pol* genes of Rous sarcoma virus³² (RSV), human adult T-cell leukaemia types I and II viruses^{33,34} (HTLV-I and -III) and Moloney murine leukaemia virus (MoMLV)³⁵. Other sequences are those of the putative polymerase of cauliflower mosaic virus³⁶ (CaMV), the product of the *pol*-like gene 17.6 (ref. 16), a transposable element and a putative polymerase encoded by hepatitis B virus³⁷ (HBV). Amino acids that are conserved in at least four sequences belonging to two different sets are boxed (only the most common amino acid at any single site is boxed). Note that many substitutions, even at the less-conserved sites, are conservative. Arrowheads point to the location of 9 of the 10 amino acids reported as invariant by Toh *et al.*¹⁴.

		I	II	III				
Mitochondrial	S.c.a1	MVLD-PKPSGEM--RPLSV	32 aa	KRM-SCGTAIW	11 aa	ILVLDKPCDITLGH	38 aa	
	S.c.a2	RVED-PKPSGCF--RPLSV	32 aa	PM-SCGTAIW	11 aa	IKVLDKPCDITLGH	38 aa	
	P.a.a1	RVVY-PKPSGEM--RPLGI	32 aa	PMR-CGSAIA	10 aa	IEGQIKGFTONTHH	37 aa	
	S.p.b1	RILU-CKASGCK--RPLTI	32 aa	PMR-SCGSAIR	11 aa	WIEGQIKGFTONTHH	37 aa	
	N.c.mau	RVVY-PKPSGEM--RPLCV	31 aa	PMR-GVFTAWR	10 aa	IYERDLKATFHSVDA	82 aa	
Retroviral	RSV	VFPV-RRASGSY--RPLHD	15 aa	QGA-PVLSALP	4 aa	LMVLDLKDCTFETPLA	25 aa	
	HTLV-I	VFPV-KKASGHW--RPLHD	15 aa	QGP-PDLSP	5 aa	LQITLDLKDCTFETPL	25 aa	
	HTLV-III	VFAK-KKASGHW--RPLVD	15 aa	QGI-PHPAGLK	4 aa	VTVLVLDLKDCTFETPL	25 aa	
	MoMLV	LLPV-KKASGHW--RPLVD	15 aa	QNYNLSALP	5 aa	VTVLVLDLKDCTFETPL	24 aa	
Other	CaMV	ERLKK--PMVNV	15 aa	PMK-DEGLT	5 aa	PSSTFELSGHGVLEDR	18 aa	
	17.6	D-ASGKKFIVID	15 aa	PMK-DEGLCKL	5 aa	ETITLDLAKGHVLEDR	18 aa	
	HBV			PMK-QSITNL	5 aa	WLSLEWYSAAYHLEPLH	72 aa	
		IV	V	VI	VII			
Mitochondrial	S.c.a1	LGTPQGLISPRIC	70 aa	YVRVADDILIC	22 aa	GLTMEKKT	11 aa	FLGYNI
	S.c.a2	LGTPQGLVSPHIC	73 aa	FVRVADDILIC	23 aa	GLSTNNKKS	10 aa	FLGYDV
	P.a.a1	IGVPGGGLASPRIC	89 aa	YVRVADDILIC	23 aa	GLTMEKKT	11 aa	FLGTEI
	S.p.b1	VGTPQGLVSPHIA	75 aa	YVRVADDILIA	22 aa	GLTMEKKT	11 aa	FLGTEI
	N.c.mau.	NGVPGGLASPRIC	13 aa	LIMVADDILIC	14 aa	GLVQPKKS	14 aa	FLGTEI
Retroviral	RSV	KVLPGQGLISPRIC	19 aa	MLRVADDILIA	21 aa	GLTMEKKT	7 aa	FLGYNI
	HTLV-I	KVLPGQGLISPRIC	19 aa	ILQVADDILIA	21 aa	GLTMEKKT	8 aa	FLGYDV
	HTLV-III	NVLPGQGLISPRIC	19 aa	IYQVADDILIA	22 aa	GLTMEKKT	7 aa	FLGYDV
	MoMLV	TRLPGQGLISPRIC	19 aa	LLQVADDILIA	21 aa	GLTMEKKT	8 aa	FLGYDV
Other	CaMV	NVYVFLGLASPRIC	14 aa	CCVYVDDILVF	21 aa	GLTMEKKT	8 aa	FLGYDV
	17.6	LRVFFGLNAPAT	15 aa	CLVYVDDILVF	21 aa	GLTMEKKT	8 aa	FLGYDV
	HBV	RKIDVGLGLSPIC	19 aa	AFVYVDDILVF	21 aa	GLTMEKKT	8 aa	FLGYDV

activity in fungal mitochondria. First, a number of *S. cerevisiae* intronic mutants revert preferentially by clean excision of the affected intron and often of one or more of its immediate neighbours¹⁷ (genetic arguments even suggested that intron-encoded proteins are involved in the process). Second, the most common form of senescence in *P. anserina* is associated with proliferation of a mitochondrial plasmid whose sequence coincides precisely with that of an intron in the cytochrome oxidase subunit I gene of this fungus¹¹. This intron is one of the four class II members discussed here.

On the other hand, the phenotypes associated with mutations in the reading frame of intron a1 of *S. cerevisiae* point to an activity other than reverse transcription. The reading frame of intron a1 continues that of the upstream exon⁵. This is the most commonly encountered situation in organelle introns and has

led to the view that their primary translation products are chimaeric proteins^{7,18,19}, another point of convergence with retroviruses and transposable elements²⁰⁻²². But although the level of expression of *pol* gene products seems to be adjusted at the translation stage (see, for example, ref. 22), synthesis of intron-encoded proteins has been postulated to be regulated at the post-transcriptional level. This is termed the 'maturase'^{7,18} or 'splicing'¹⁹ model, according to which chimaeric protein translated from a pre-mRNA molecule (or its proteolytic derivatives, see Fig. 2) contributes to the destruction of its own mRNA by participating in the splicing process (in this model it is assumed that the excised form of the intron, which, in the case of a1, is a stable circular RNA²³, does not give rise to a functional translation product). Several *trans*-recessive splicing-deficient mutations have been identified that map within the open reading frame of intron a1 and block its excision²⁴.

However, that the protein encoded by intron a1 should be involved in excision of the intron does not contradict the fact that part of it is related to reverse transcriptases. Several structured introns catalyse their own excision from primary transcripts without the need for a protein helper^{25,26} (the best-known example is provided by the *Tetrahymena* ribosomal intron, one of the few nucleus encoded class I introns^{1,27,28}). Thus, as far as splicing is concerned, maturases seem to be accessory devices and maturase activity could have evolved from intron-associated proteins as a form of self-regulation. On the other hand, possession of a specialized reverse transcriptase, although of no normal use in organelles, could be of decisive advantage in the maintenance and propagation of structured introns in the genomes of organelles. Thus, it will be of interest to determine whether the proteins encoded by class II introns have a DNA endonuclease activity similar to that believed to be involved in integration of retroviral DNA into the host genome²⁹⁻³¹, which is encoded by the 3' section of *pol* genes (see Fig. 2).

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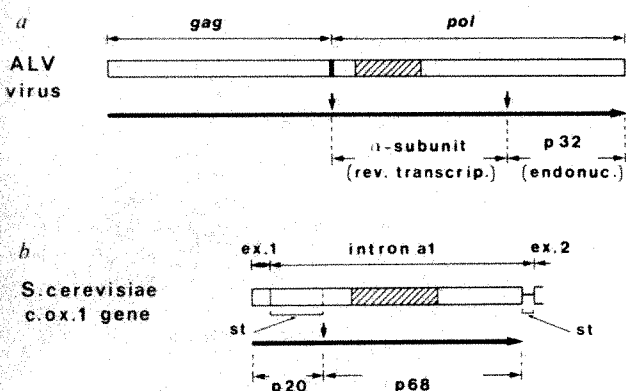


Fig. 2 Organization and expression of the *pol* gene of avian sarcoma-leukosis virus (ALV) compared with those of intron a1 in the cytochrome oxidase subunit I gene of *S. cerevisiae*. *a*, The gene and its products, redrawn from ref. 38; *b*, intron a1, redrawn from ref. 24. Homologous sections in the two genes (see Fig. 1) are hatched. Primary translation products are sketched as thick lines with an arrow-shaped extremity that indicates the direction of translation. Vertical arrows point to proven or presumed sites of proteolytic cleavage; st, sections of intron a1 that participate in class II secondary structure ('core' of the intron, see ref. 2). In the case of intron a1, the active product has been proposed to be p68 (ref. 24), a polypeptide which seems to correspond to most or all of that part of the intronic reading frame that is looped out of the structured core of the intron by long-range potential base pairings, also well conserved in evolution^{2,12}.

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Haemophilia B caused by a point mutation in a donor splice junction of the human factor IX gene

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Haemophilia B (Christmas disease) is an inherited, recessive, sex-linked, haemorrhagic condition caused by a defect in the intrinsic clotting factor IX. This disease occurs in males at a frequency of ~1 in 30,000. Patients differ in the severity of their clinical symptoms, and variation in the clotting activity and in the concentration of factor IX antigen in their plasma has been demonstrated¹. There is probably heterogeneity in the molecular defects of the factor IX gene causing the disease. Here we study a severely affected, antigen-negative patient, and show that the only significant sequence difference from the normal factor IX gene is a point mutation changing the obligatory GT to a TT within the donor splice junction of exon f. We infer that this change is the cause of the disease in this individual. In addition, we have used oligodeoxynucleotide probes specific for this mutation to demonstrate the feasibility of carrier detection and prenatal diagnosis for relatives of the patient.

The characterization of the normal factor IX gene² made it possible to characterize haemophilia B at a genetic level. Approximately 40% of patients have a normal concentration of factor IX antigen (IXAg) in their plasma³ and probably have point mutations in the coding sequence giving rise to single

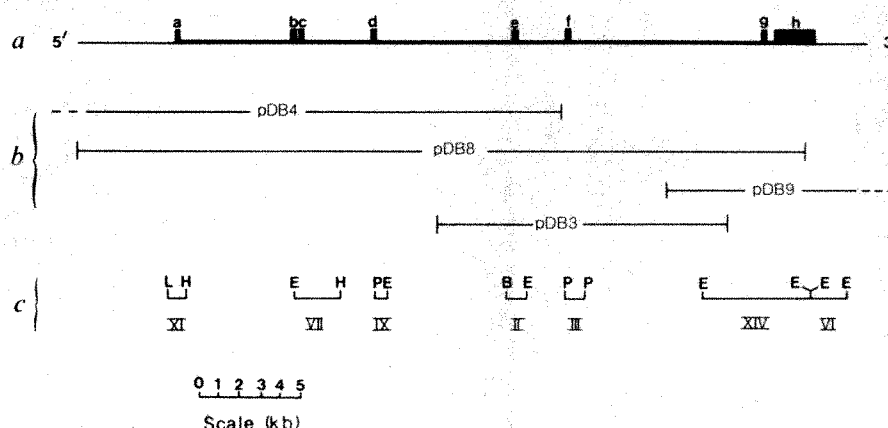
amino-acid changes such as that found in factor IX^{Chapel Hill} (ref. 4). A few patients (1%) produce antibodies to the injected factor IX used in replacement therapy and here the defect appears, in most cases, to be large deletions in the factor IX gene⁵. The remaining 60% of patients have either markedly reduced or undetectable levels of IXAg and can be described as cross-reactive-material negative (CRM negative)¹. It is plausible that the defect in this group of patients could be either mutations resulting in a reduction in the amount of factor IX messenger RNA, or chain termination mutations causing the production of truncated and presumably unstable factor IX protein.

The patient (D.B.) chosen to test this hypothesis falls into the severe, CRM-negative class, having 0.3% factor IXAg⁶ (assayed by radioimmunoassay⁷) and <0.5% factor IX clotting activity⁶ (assayed by two-stage thromboplastin generation assay⁸). A genomic cosmid library was prepared from DNA isolated from the patient's peripheral blood lymphocytes and cloned in the cosmid vector pTM (ref. 9). Four recombinant clones containing factor IX sequences were isolated and mapped as before² (Fig. 1). From these clones, all eight exon regions were separately subcloned and sequenced¹³, except for that part of exon h corresponding to the 3' noncoding section of the mRNA. The sequences determined totalled 5,200 bases, covering the presumptive promoter, the 5' noncoding region of the mRNA, all protein-coding sequences and the polyadenylation signal AATAAA.

The sequence was found to be identical to the previously published normal factor IX gene² with 14 exceptions. A G→T change was found at position 21,165, within the obligatory GT of the donor splice site at the 3' site of exon f (Fig. 2), which changes an *RsaI* site in the normal sequence to a *MaeIII* site

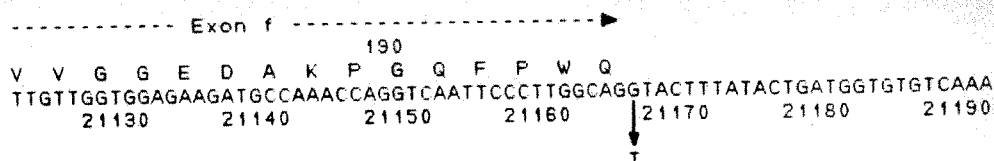
Fig. 1 Line diagram of the positions of clones isolated from patient D.B. in relation to the organization of the factor IX gene. *a*, The position of the eight exons *a-h*; *b*, the position of cosmid clones isolated from patient D.B.; *c*, the subclones generated for sequencing.

Methods. DNA was isolated¹⁰ from peripheral blood lymphocytes from 100 ml of whole blood. It was partially restricted with *MboI* and size fractionated on a 1-5 M NaCl gradient⁹. DNA in the size range 35-45 kb was pooled, ligated into the cosmid vector pTM⁹, packaged into bacteriophage λ heads¹⁰ and allowed to infect *Escherichia coli* ED 8767 (ref. 11). 1.5×10^6 clones were screened by colony hybridization¹² using previously isolated factor IX probes XI,



IV and cVII (ref. 2). Positive clones were mapped by digestion with restriction enzymes to the positions shown by comparison with maps of previously characterized genomic clones. Clone pDB3 contains approximately 15 kb of the factor IX gene ligated to approximately 20 kb from elsewhere in the genome as a result of a cloning artefact. Subclones constructed for sequencing were derived by restriction digestion of the appropriate cosmid clone and isolation of the required fragments, which were "filled in" with deoxynucleoside triphosphates using the Klenow fragment of *E. coli* DNA polymerase I¹⁰ and blunt-end ligated into the vector pATX (C. Naeve, unpublished) except for subclones XIV and VI. These were ligated directly into the *EcoRI* site of pBR322. The restriction sites at the ends of each subclone are: B, *BglII*; E, *EcoRI*; H, *HindIII*; L, *BglII*; P, *PvuII*. Subclones are numbered as before².

Fig. 2 The sequence surrounding the 3' end of exon f of the normal factor IX gene showing the position of the G→T mutation found in the donor splice site in patient D.B. The other sequence changes found in D.B. were G→C at position 6,928 at a previously discovered polymorphic *Xmn*I site¹⁴, T→C at position 34,037 and A→G at an unmapped position not more than 1.4 kb from the 3' end of the gene. Nine other differences reflected sequence errors in the normal factor IX gene sequence and corrections to Fig. 4 of ref. 2 are as follows: 138 = G, 139 = A, 309 = T, 310 = C, 319 = A, delete residues 6,050, 34,026 to 34,031 and 34,093, insert T between residues 34,107 and 34,108 and insert T between residues 34,170 and 34,171. (Fig. 2 of ref. 2 should also be corrected at the three relevant positions.)



in the patient. A G→C change was found at position 6,928 resulting in the loss of an *Xmn*I site previously shown to be polymorphic in normal populations¹⁴ (see Fig. 2 legend). Two further differences were found 3' to the factor IX gene (see Fig. 2 legend), and these may also be polymorphic but do not result in the change of any restriction enzyme sites. The 10 remaining differences were all caused by errors in the normal factor IX sequence² (see Fig. 2 legend).

To test the feasibility of directly assaying the molecular defect in total DNA isolated from peripheral blood lymphocytes, we synthesized oligodeoxynucleotide probes¹⁵, designed according to the principles developed to detect point mutations in globin genes¹⁶, to analyse this mutation in the patient and his mother (Fig. 3). Two 19-base long oligodeoxynucleotides (probes 1 and 2; see Fig. 3 legend) were used to hybridize to uncloned and cloned sequences under conditions in which only exactly matching sequences hybridized¹⁶. Probe 1 (exactly complementary to the normal factor IX sequence) hybridizes to a 1.1-kilobase (kb) *Pvu*II restriction fragment² in digests of DNA isolated from a normal male, from a normal female, from a cell line with 4X chromosomes (GM1416) and from the patient's mother (M.B.) (Fig. 3B a-c, e), as well as in *Pvu*II-restricted cloned normal factor IX DNA (Fig. 3B g). Thus, probe 1 hybridizes to the correctly sized restriction fragment and with the intensity expected

for an X chromosomal sequence (that is, with a ratio of intensities of about 1:2:4 on 1X, 2X and 4X genomic DNA). In addition, probe 1 fails to hybridize to the mutant sequence either in uncloned or cloned DNA (Fig. 3B d, f).

Probe 2 (exactly complementary to the mutated sequence in the patient) hybridizes to the same restriction fragment only in *Pvu*II digests of DNA isolated from the patient, his mother and the cloned mutant factor IX gene (Fig. 3B k-m) and fails to hybridize to the normal sequence (Fig. 3B h-j, n). Thus, these probes differentiate specifically between the normal and mutant sequences and in addition confirm that the base change characterized is not a cloning artefact. Both probes also hybridize to the DNA isolated from the patient's mother, in agreement with her obligatory carrier status (Fig. 3). If DNA were available from II₅ and II₆, it would be possible to determine their carrier status. If they are carriers, both probes 1 and 2 would hybridize whereas if not, only probe 1 would hybridize. It would also have been possible to use digestion with *Rsa*I and Southern blot analysis to assay the molecular defect directly. This should show a patient-specific fragment of 1.3 kilobases (kb) when probed with probe III², compared with a normal fragment of 1.1 kb.

We then analysed the DNA from nine other unrelated CRM negative patients whose factor IX antigen levels ranged from 1 to 6% of normal in a radioimmune assay^{6,7}. Under identical hybridization conditions to those used above, all hybridized to probe 1 (Fig. 4) and failed to hybridize to probe 2 (results not shown), indicating that they had the normal sequence at this position. Presumably the defect in these patients occurred elsewhere in the factor IX gene, either in other splice donor or acceptor sequences or other sequences essential for normal gene expression, as has been found to be the case in α and β thalassaemias¹⁷.

We have shown that a point mutation (G→T) within the obligatory GT of a donor splice junction is the only significant sequence change in the factor IX gene from patient D.B. It is well established that this dinucleotide is highly conserved¹⁸ and essential for the normal splicing of pre-mRNA to mRNA so that any alteration of this sequence would be expected to perturb this process. Perhaps the most convincing supporting evidence is that mutations in the GT of donor splice sequences in th

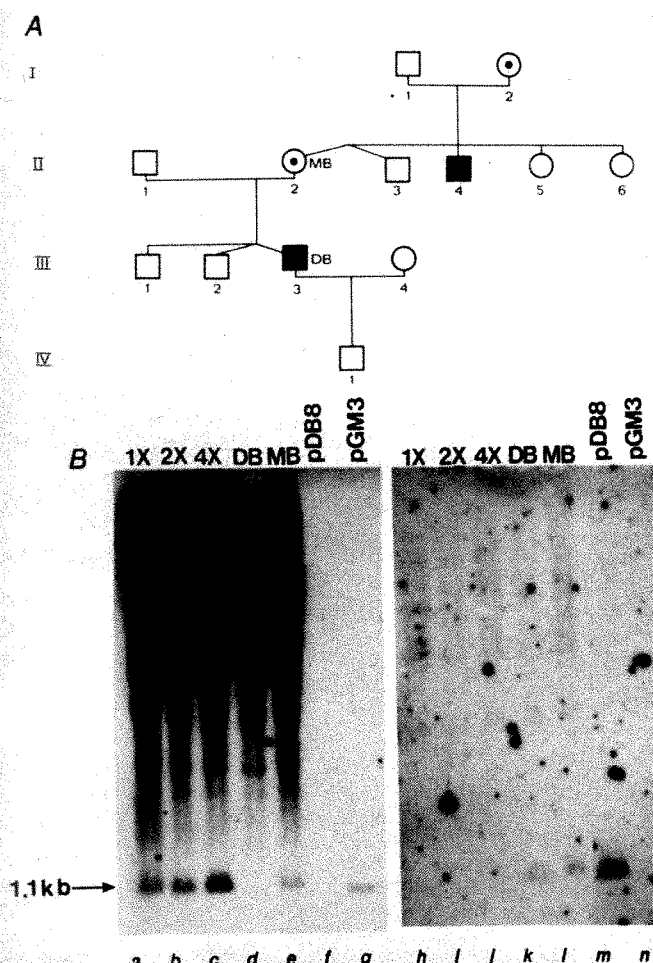


Fig. 3 **A**, family tree of the D.B. family showing the inheritance of haemophilia B. ■, Affected male; ○, obligatory carrier. The status of II₅ and II₆ is unknown. **B**, oligonucleotide hybridization studies. Two oligonucleotide probes were synthesized¹⁵, being complementary to residues 21,157-21,175. Probe 1 is the normal sequence 5' GTATAAAGTACCTGCCAAG 3' and probe 2 is the mutated sequence 5' GTATAAAGTAACTGCCAAG 3', differing from probe 1 at position 11. These were used for hybridization studies to genomic and cloned factor IX sequences as shown. a-e, h-l contain 15 µg of the indicated genomic DNA; f, m, 0.5 ng and 10 ng respectively of pDB8; g, n, 0.5 ng and 10 ng respectively of pGM3 (a cosmid clone, containing the 5' half of the normal factor IX gene, D.J.G.R., unpublished). DNA samples were digested to completion with *Pvu*II and electrophoresed in a 20-cm 0.7% agarose gel in 90 mM Tris, 90 mM boric acid, 2.5 mM EDTA, pH 8.3. The gel was then treated as described by Conner *et al.*¹⁶. 50 ng of each oligonucleotide was labelled with [γ -³²P]ATP using T4 polynucleotide kinase (Amersham) to a specific activity of 10⁹ d.p.m. µg⁻¹ and hybridized to the dried gel for 16 h at 42 °C. The gels were then washed as described, with a final temperature of 60 °C in 6 × SSC¹⁶.

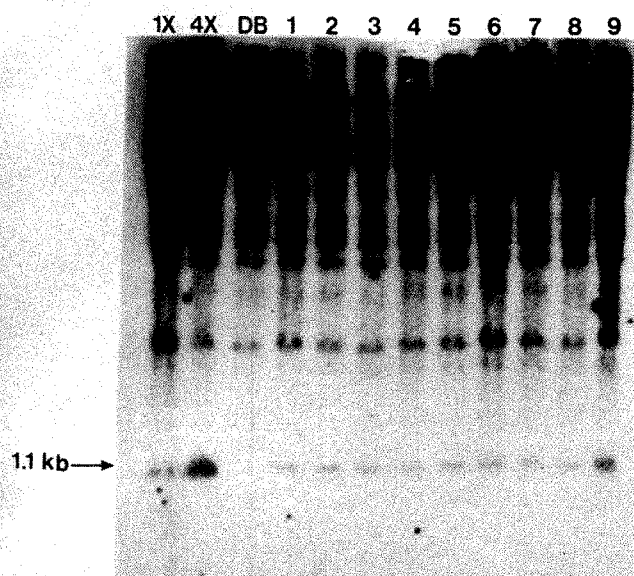


Fig. 4 Hybridization of ^{32}P -labelled probe 1 to DNA (15 μg per track) from nine CRM-negative haemophilia B patients from unrelated kindreds⁶ (tracks 1-9). The samples were digested, electrophoresed in 0.7% agarose, hybridized and washed as described in Fig. 3. 1X and 4X DNA samples (15 μg) were included as positive controls; DNA (15 μg) from the patient D.B. was used as a negative control. Hybridization of probe 2 under identical conditions showed a positive signal only on the track containing DNA from D.B. (data not shown).

β -globin gene are known to cause β^0 thalassaemias with no production of cytoplasmic β -globin mRNA¹⁷. We have been unable to study the effect of this mutation on the *in vivo* splicing of the factor IX pre-mRNA in our patient because of the difficulty of obtaining liver biopsy samples, which are the source of the hepatocytes that express factor IX. However, in theory, if the incompletely spliced mRNA were translated and the protein processed normally, a truncated protein of 217 residues would be produced which extends 22 amino-acid residues from exon 9 and terminates at TGA at nucleotide 21,231 (ref. 2). In practice, there is doubt that this occurs because virtually no factor IX antigen is detectable in plasma by radioimmunoassay.

It has been possible in both α and β thalassaemias to use oligodeoxynucleotide probes to detect single base changes in genomic DNA. Here we have shown that this approach can be successfully applied to haemophilia B, both to detect a single base change in the patient and to detect the presence of the mutant allele in a heterozygote. This should allow the use of mutation-specific probes to perform prenatal and carrier diagnosis of haemophilia B in families carrying mutations that have been characterized at the molecular level. In addition, using the same techniques, it should be possible to analyse naturally occurring sequence differences such as those characterized here to the factor IX gene, which do not alter restriction enzyme cleavage sites in genomic DNA, to determine whether they are polymorphic in the normal population. If this is the case, then their use in linkage analysis should extend the range of carrier and prenatal diagnostic facility currently available for haemophilia B^{14,19}.

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Attachment of transported vesicles to microtubules in axoplasm is facilitated by AMP-PNP

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Axoplasm extruded from the squid giant axon has been used to analyse the molecular mechanisms of intracellular vesicle transport^{1,2}. Using video-enhanced light microscopy, vesicle transport can be observed directly on individual microtubules at the edge of the axoplasm²⁻⁴. Here we report that AMP-PNP (adenyl-5'-yl imidodiphosphate), a non-hydrolysable analogue of ATP, reversibly inhibited vesicle transport. Moreover, vesicles in solution attach to the microtubules and form relatively stable complexes. AMP-PNP may produce this effect by binding to an ATP-binding site on the transport machinery, thereby stabilizing the motility complex that is normally formed by a transported vesicle, an ATPase and a microtubule. The effects of AMP-PNP on the vesicle transport system indicate that the enzymatic machinery of this system differs significantly from that of the actomyosin system or the dynein-microtubule system.

Using the extruded axoplasm model, we have begun to explore the following question: is the molecular mechanism of vesicle transport similar to the two well-characterized models of cell motility in metazoa, the actomyosin-based system in muscle^{5,6} and the dynein-based system in the axonemes of flagella and cilia^{7,8}? One basic property of both these systems is their response to changes in the concentration of ATP. When ATP is absent, myosin and dynein form stable (rigor) complexes with their motile partner (actin and the B subfibril, respectively)⁵⁻⁸. By contrast, when ATP is added to a muscle or an axoneme in rigor, the rigor complexes rapidly dissociate and the ATP is hydrolysed⁶⁻¹⁰.

Non-hydrolysable analogues of ATP, such as AMP-PNP, can be used selectively to block events in the cycle of motility which are dependent on the hydrolysis of ATP¹¹. In muscles and axonemes, AMP-PNP stabilizes those transient intermediates that occur in the motility cycle just before ATP hydrolysis⁶⁻¹⁴. AMP-PNP acts by binding specifically to the ATP binding site on myosin and dynein; and, like ATP, it tends to dissociate the actomyosin and dynein-B subfibril complexes *in vitro*¹⁰⁻¹⁴. However, myosin and dynein have a lower affinity for AMP-PNP than for ATP, and AMP-PNP is less effective than ATP at dissociating the rigor complexes¹⁰⁻¹⁴.

We have used AMP-PNP to obtain information about the transient intermediates that are formed before ATP hydrolysis in the motility cycle of vesicle transport. Perfusion of axoplasm with a physiological buffer (see Fig. 1 legend) containing 0.5-

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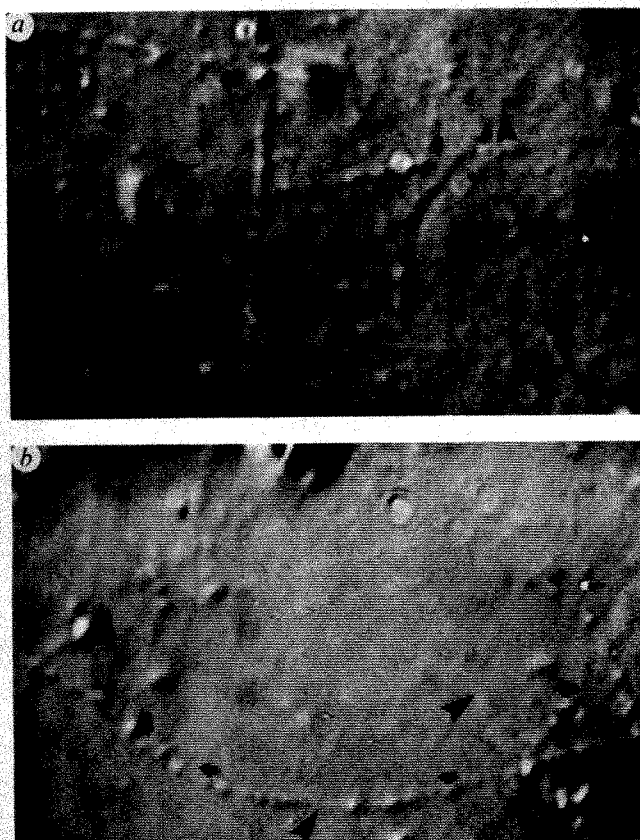


Fig. 1 Microtubules at the periphery of the axoplasm (arrowheads). *a*, Isolated microtubules in buffer X without AMP-PNP. Vesicles in solution frequently attach and translocate along the microtubules. Arrows, examples of vesicles moving along microtubules. *b*, Microtubules in the presence of 5 mM AMP-PNP (lithium salt). In the presence of AMP-PNP, vesicles attach to the microtubules and remain stationary, producing the appearance of a 'string of pearls'. Small, medium and large vesicles are indicated by different sized arrows. In parallel experiments, Li^+ at concentrations of 1–5 mM had no effect.

Methods. In all the experiments described here, axoplasm was obtained from the medial third-order giant fibres of squid (*Loligo pealei*). Briefly, the giant fibres were tied off at each end and removed from the animal^{2,20}. After removing excess connective tissue, the fibre was rinsed in buffer X (350 mM potassium aspartate, 130 mM taurine, 70 mM betaine, 50 mM glycine, 20 mM HEPES, 12.9 mM MgCl_2 , 10 mM EGTA, 3 mM CaCl_2 , 1 mM glucose, 1 mM ATP, pH adjusted to 7.2 with KOH), blotted dry and the proximal end cut². A cylinder of axoplasm was extruded onto a coverslip, which formed the lower surface of the observation chamber². To form the side walls of the chamber, two thin spacers were placed on either side of the axoplasm. A space of ~1 mm was left between each spacer and the axoplasm, and a coverslip with spacers was gently placed on top of the axoplasm to complete the chamber. All the experimental observations began with a scan of the axoplasm to ensure that the preparation was active; then 10 μl buffer X containing 0–1 mM ATP and 0–10 mM AMP-PNP was perfused through the chamber. Movement was observed in the centre and at the periphery for 30 min or until no movement was observed. Video-microscopy was carried out using AVEC-DIC (video-enhanced differential interference contrast) microscopy using a Zeiss Axiomat microscope and Hamamatsu video equipment^{1,3}.

10 mM AMP-PNP immediately blocked the movement of vesicles on microtubules at the periphery of the axoplasm (Fig. 1). Below 0.5 mM AMP-PNP, movement was not effectively blocked.

At AMP-PNP concentrations which blocked transport, free vesicles in the solution attach normally to the peripheral microtubules but do not translocate. After 3–5 min, so many vesicles had attached to the microtubules and then remained stationary that the microtubules had the appearance of a 'string of pearls' (Fig. 1b).

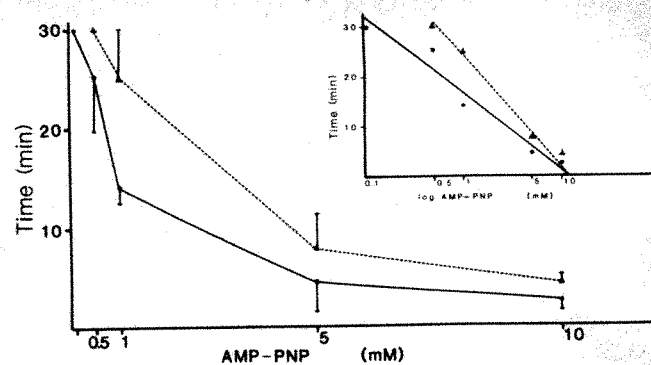


Fig. 2 Effects of ATP and AMP-PNP in the presence and absence of exogenous ATP. Axoplasm was extruded as described in Fig. 1 legend. A known volume of buffer, which contained either AMP-PNP alone (circles) or AMP-PNP with 1 mM ATP added (triangles and dashed line), was perfused into the chamber. The ratio of the buffer volume to axoplasm volume was ~5:1. Vesicle movement was observed in the centre of the axoplasm and recorded every 5 min until either >95% of the vesicles had stopped moving or 30 min had elapsed. The results are plotted as a function of increasing concentration of AMP-PNP against time taken for inhibition of vesicle movement. All experiments were done in replicate with $n = 2$ –4 for each condition; vertical bars indicate s.e.m. The difference between the time to inhibition with AMP-PNP alone and with AMP-PNP + 1 mM ATP is significant for all concentrations tested at $P > 0.01$. The inset shows the same data plotted against the log of the concentration of AMP-PNP. Lines on the inset were fitted by the linear regression method and each line had a regression coefficient of $r = 0.94$. These dose-response experiments indicate that it takes ~25 min for the lowest effective concentrations of AMP-PNP (0.5 mM) to inhibit reproducibly vesicle movement in the centre of the axoplasm. Presumably, this represents the interval required for the concentrations of AMP-PNP and ATP to equilibrate between the axoplasm and the external buffer because vesicle movement stops immediately at the periphery of the axoplasm.

In the central axoplasm, vesicle movement was also inhibited by AMP-PNP². At 10 mM exogenous AMP-PNP, levels of AMP-PNP in the centre of the axoplasm reached a sufficient concentration to inhibit vesicle movement after only 2 min. At lower concentrations complete arrest of vesicle movement in the centre of the axoplasm was delayed by the time that it takes for sufficient AMP-PNP to diffuse from the external buffer into the axoplasm (Fig. 2).

AMP-PNP inhibited vesicle movement at concentrations as low as 0.5 mM, which is similar to the ATP concentration in the axoplasm. Normally, axoplasm contains ~1 mM endogenous ATP but when the axoplasm and buffer in the perfusion chamber equilibrate, the final concentration of ATP is ~0.2 mM. This concentration of ATP is sufficient to maintain normal vesicle transport for the duration of the experiment. Thus, AMP-PNP completely inhibited particle movement in the presence of ATP at a concentration that normally maintains motility. By contrast, AMP-PNP does not completely inhibit motility in muscle and axonemes when the concentration of ATP is sufficient to maintain motility.

AMP-PNP may block vesicle transport by effectively competing with ATP for the nucleotide binding site or sites in the vesicle transport machinery. To test this hypothesis, we examined the effects of adding exogenous ATP on the inhibition of vesicle movement by AMP-PNP (Fig. 2). We found that 1 mM ATP significantly delayed the inhibitory effect of AMP-PNP at all concentrations and completely blocked the inhibitory effects of 0.5 mM AMP-PNP. Moreover, the inhibitory effect of AMP-PNP on vesicle transport could be reversed by perfusion with excess ATP.

In the ATP reversal experiments, the observation chamber was first perfused with 1 mM AMP-PNP to inhibit vesicle movement and then perfused twice with 10 μl buffer containing 10 mM ATP. Vesicle movement resumed at the periphery within

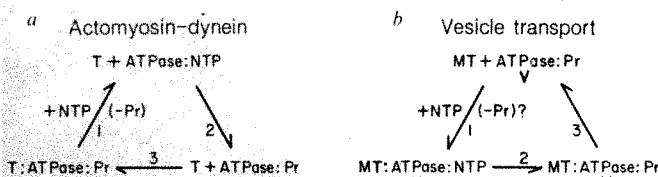


Fig. 3 Schematic enzyme mechanisms for intracellular motility cycles⁷⁻⁹ comparing the actomyosin and dynein ATPase cycles (a) with the vesicle transport cycle (b). a, 'ATPase' refers to either myosin or dynein and T refers to the translocatable partners (that is, filamentous actin or the B subfibril). NTP refers to nucleotide (for example, ATP or AMP-PNP) and Pr refers to the products of ATP hydrolysis. The drive stroke for myosin and dynein occurs after step 3. b, 'ATPase' refers to the putative ATPase in the crossbridge that mediates the attachment of the translocatable vesicles (V) to the microtubules (MT)¹⁵. We propose that ATP modulates the affinity of the ATPase-vesicle complex for the substrate microtubule. This hypothesis is supported by the observation that vesicles prepared from squid axoplasm retain their motile ATPase and can translocate along flagellar microtubules¹⁷. For simplicity the scheme is drawn as if the vesicle transport ATPase has a stable association with the vesicle. However, the ATPase can apparently dissociate from the vesicle. The step at which products (Pr) are released remains to be determined in both (a) and (b). Note that the effect of the binding of nucleotide (NTP) in the actomyosin and dynein cycles (a) is the opposite of its effect in the vesicle-transport cycle (b).

1-2 min of ATP addition, and vesicles began to move in the centre of the axoplasm 10 min after ATP addition.

Based on these results, we propose that AMP-PNP operates by competitively attaching to a critical ATP binding site in the motility machinery of vesicle transport. Miller and Lasek¹⁵ have recently proposed that the critical ATP binding site is an ATPase that is located on the 16-18-nm-long crossbridges that attach the transported vesicle to its substrate microtubule. Thus, like the actomyosin system and the dynein-microtubule system, motility in the vesicle transport system may be based on an ATP-dependent crossbridge cycle.

ATP has two important functions in the crossbridge cycle of muscles and axonemes⁷⁻⁹; it provides energy for movement and reduces the affinity of the crossbridge (that is, myosin or dynein) for its motile partner (that is, actin or tubulin) (Fig. 3a). In muscle, the biochemical cycle producing movement begins with the binding of ATP to the stable actin-myosin (rigor) complex⁹ which induces a rapid release of the myosin crossbridge from actin. Myosin hydrolyses the ATP, producing an activated ADP-myosin complex that has a much higher affinity for actin than the ATP-myosin complex. When the activated ADP-myosin complex rebinds to actin, it releases its stored energy in the motile power stroke. A similar scheme has been proposed for axonemes^{7,8}.

The effects of AMP-PNP on vesicle transport suggest that the enzymatic cycle of vesicle transport differs in at least two important ways from the cycle proposed for muscle and axonemes. First, dynein and myosin ATPases are weakly inhibited by AMP-PNP, that is, AMP-PNP will not produce complete inhibition of the biochemical cycle at concentrations of ATP that support motility^{6,14}. By contrast, the vesicle transport ATPase is completely inhibited by AMP-PNP even in the presence of concentrations of ATP that readily support vesicle transport. Second, in both muscles and axonemes, nucleotide binding promotes the dissociation of the motility complex (Fig. 3a, step 1)^{6,10,13}. In contrast, AMP-PNP facilitates a stable association of vesicles with microtubules. This suggests that nucleotide binding (for example, ATP or AMP-PNP) to the vesicle-transport ATPase promotes the formation of the basic motility complex (the vesicle-crossbridge-microtubule complex; Fig. 3b, step 1), which is the opposite of the effect of nucleotide on myosin and dynein (Fig. 3a).

The scheme proposed for the vesicle-transport cycle in Fig. 3b is further supported by observations of vesicle movement in ATP-depleted axoplasm. When ATP is eliminated from squid axoplasm by conversion to AMP and pyrophosphate with the

enzyme apyrase, vesicle transport is inhibited and most vesicles are subject to a constrained brownian motion². Apparently, rigor complexes are not favoured in the vesicle-transport system in low ATP concentrations.

The structural organization of the vesicle transport system may be analogous in some respects to that of the actomyosin and the dynein-microtubule systems¹⁵⁻¹⁷. Significantly, in these three systems a crossbridge is the structural link between the stationary substrate and the translocated partner^{5,8,15}. However, although there may be structural analogies between these motile systems, the results presented here and others^{18,19} indicate that the enzymatic properties of the motile protein of vesicle transport differ significantly from the crossbridge proteins in both muscle and axonemes (that is, myosin and dynein, respectively).

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Primary structure of bovine thyroglobulin deduced from the sequence of its 8,431-base complementary DNA

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In mammals, an adequate supply of thyroid hormones is essential for normal growth and neurological development. The biosynthesis of thyroid hormones involves an iodinated precursor protein, thyroglobulin, which may be considered an extreme example of a pro-hormone¹. Thyroglobulin is a dimeric glycoprotein of relative molecular mass (M_r) 660,000 (660K), which is secreted by the thyrocyte and stored in the lumen of the thyroid follicle. The hormonogenic reaction is extracellular, and involves iodination of tyrosyl residues of thyroglobulin and the intramolecular coupling of a subset of these into thyroxine (T_4) and triiodothyronine (T_3), which remain part of the polypeptide chain. Secretion of hormones results from the endocytosis of thyroglobulin followed by its complete hydrolysis in lysosomes. Considering that the maximum yield of hormones is ~6-8 per 660K protein², the whole process is apparently wasteful. However, the efficiency of thyroglobulin as a thyroid hormone precursor is extremely high when the supply of iodine is short; in such conditions, almost all the iodine incorporated is found in iodothyronines³. Hence it is suggested that the thyroglobulin structure has evolved to allow for the preferential and efficient iodination and coupling of the hormonogenic tyrosines. Here we report the complete primary structure of bovine thyroglobulin, derived from the sequence of its 8,431-base-pair complementary DNA. The 2,769-amino-acid sequence is characterized by a pattern of imperfect repeats derived from three cysteine-rich motifs. Four hormonogenic tyrosines have been precisely localized near the amino and carboxyl ends of the protein.

The complete nucleotide sequence of bovine thyroglobulin messenger RNA (Fig. 1) was obtained by sequencing six recombinant plasmids containing 99% of its cDNA⁴ and one genomic

[illegible]

Fig. 1 Sequence of thyroglobulin cDNA and the deduced primary structure of the protein. Nucleotides are numbered (on the left of the figure) from the ATG initiation codon. The deduced amino acids are numbered from the amino-terminal asparagine of the mature protein. The polyadenylation signal⁸ is underlined. The putative *N*-glycosylation sites (Asn-X-Ser/Thr) and the tyrosine residues involved in hormonogenesis are underlined and overlined, respectively.

Methods. Six recombinant plasmids⁴ and one genomic DNA fragment (G. de Martynoff *et al.*, in preparation) were used as starting material. Subcloning in phage M13 vectors²⁴ and generation of overlapping fragments with DNase were performed as described in ref. 25. The dideoxy chain termination method²⁶ and buffer gradient gels²⁷ were used for DNA sequence analysis. Confidence was obtained by reading each nucleotide on both DNA strands. The overlapping recombinant plasmids⁴ differ at two positions: nucleotide 4,585 is A in pbTg 1.9 and G in pbTg 4.7 (changing amino-acid 1,510 from Ser to Gly), and nucleotide 7,141 is G in pbTg 4.7 and A in pbTg 2.5 (changing amino-acid 2,362 from Val to Met). These differences may reflect sequence polymorphism. However, an error in the reverse transcriptase reaction cannot be excluded.

However, an error in the reverse transcriptase reaction cannot be excluded.

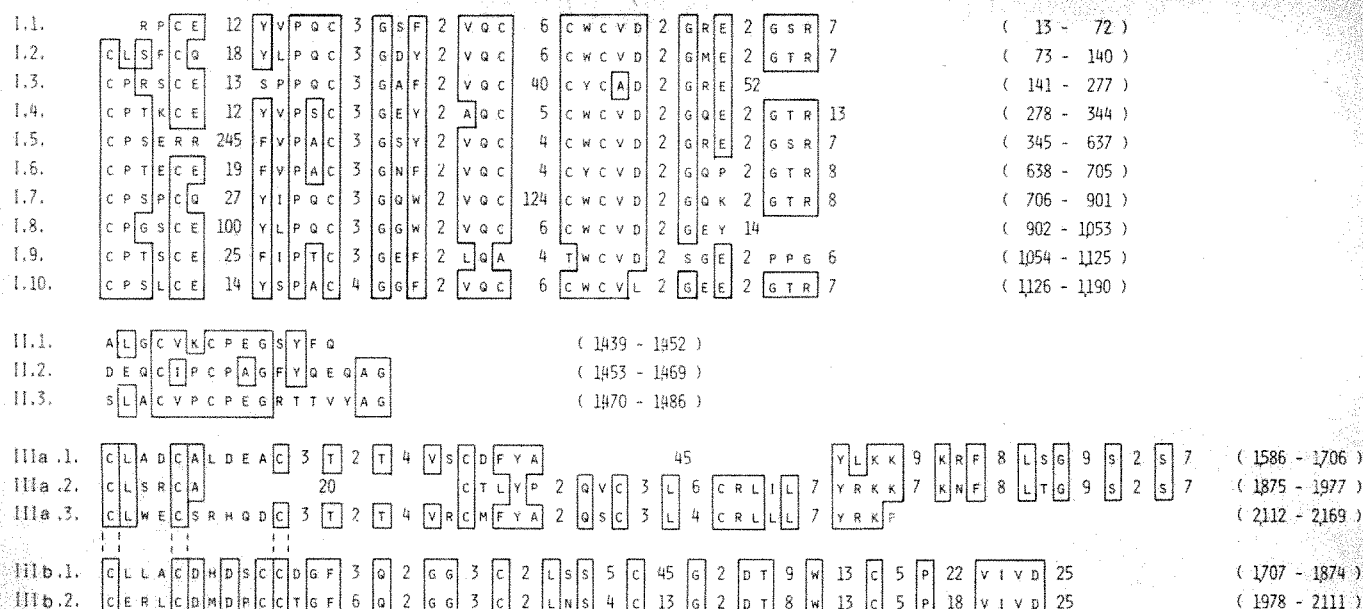


Fig. 2 Internal homology in thyroglobulin primary structure. The three types of homologous domains (given in the one-letter code) are aligned. Insertions or deletions have been introduced to maximize homology. Conserved amino acids are boxed according to the following rule: for type I repeats, the amino acids should be present at least five times; for type II and III repeats, at least twice. Residues showing no conservation between the domains are represented by numbers. Homologies in Cys positions between domains IIIa and IIIb are indicated by broken lines. Localization of the domains is shown on the right; the numbering is as in Fig. 1.

DNA fragment containing the 5'-terminal portion of the message (G. de Martynoff *et al.*, in preparation). Partial nucleotide sequences have been reported for three species⁵⁻⁷ but they do not exceed 3,000 bases long. The general organization of the mRNA sequence is as follows: a 41-nucleotide 5'-untranslated segment precedes an open reading frame of 8,307 bases. To our knowledge, this represents the longest coding sequence determined to date. The 3'-untranslated segment is 83 bases long and contains the canonical AATAAA polyadenylation signal⁸.

As judged from the nucleotide sequence, a 19-amino-acid signal peptide is followed by 2,750 residues, constituting a polypeptide of expected M_r 302,253; this value is in perfect agreement with the M_r obtained for the translation product of thyroglobulin mRNA *in vitro*⁹ or in *Xenopus* oocyte¹⁰ and with the M_r of 330,000 for the glycosylated protomer¹¹. The amino-acid composition of the protein agrees with published results (not shown). In particular, despite their role in hormonogenesis, the number of tyrosine residues (72, that is, 2.5%) does not deviate significantly from the average observed in a protein data bank (3.3%; ref. 12). The sequence contains 15 potential acceptor sites for *N*-glycosylation (Fig. 4), which represents a slight excess compared with the 20 chains characterized in the mature dimeric protein¹³.

When analysed for the presence of internal homology, the protein sequence shows a striking repetitive pattern involving three types of cysteine-containing motifs (Fig. 2). The type I motif is repeated 10 times between positions 13 and 1,190; it extends over ~60 amino acids and contains 24 highly conserved residues, including six cysteines. Sequences of variable length, unrelated to the motif, interrupt some of the repeats at fixed positions (Fig. 2). Preliminary data indicate that the positions of these insertions correlate with the borders of exons (J. Parma, in preparation). The type II motif is much shorter (17 amino acids) and is only repeated three times between positions 1,439 and 1,486. The type III motif may be subdivided into two subtypes (IIIa, IIIb; Fig. 2), which display a similar pattern of cysteine residues. Both subtypes are repeated twice in tandem, between positions 1,586 and 2,111. A third copy of motif IIIa is found further downstream (Fig. 4). Over 75% of the thyroglobulin sequence is thus involved in some kind of repetitive structure, clearly indicating that this exceptionally large protein evolved from the serial duplication of a limited number of building blocks. Interestingly, the last 600 residues at the carboxyl end show no internal homology, are poor in cysteine and contain a cluster of tyrosines of which three are hormonogenic (see below and Fig. 4).

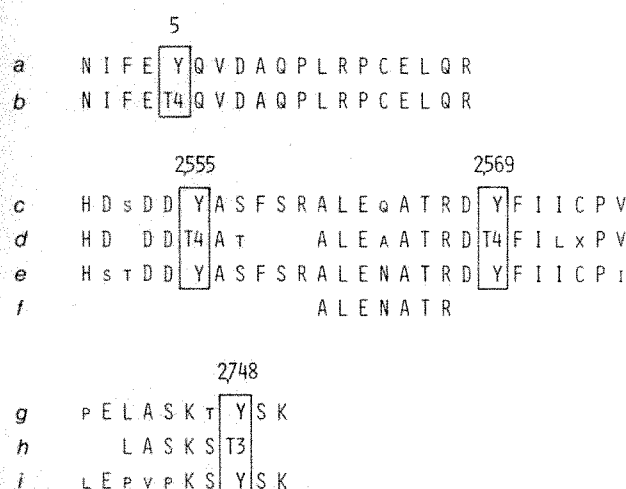


Fig. 3 Hormonogenic domains in thyroglobulin. The hormone-containing domains identified to date in various species are represented by the one-letter code and are aligned for maximum homology. The capital letters correspond to amino acids occurring at least twice in different species. The four hormonogenic tyrosines are boxed and their positions in the protein are indicated. *a*, *c*, *g*, the peptide sequences derived from the present study; *b*, the four identical hormone-containing peptides determined from bovine, human, ovine and porcine thyroglobulin^{17,28,29}; *d*, the sequence of three peptides obtained from porcine thyroglobulin, named *b*₁Ad₃, T6 and *b*₁B in ref. 18; *e*, *i*, protein sequence derived from a partial rat cDNA sequence⁷; *f*, a tryptic peptide, isolated from human thyroglobulin, containing a glycosidic chain attached to the asparagine residue³⁰; *h*, sequence of a hormone-containing peptide isolated from porcine thyroglobulin¹⁹.

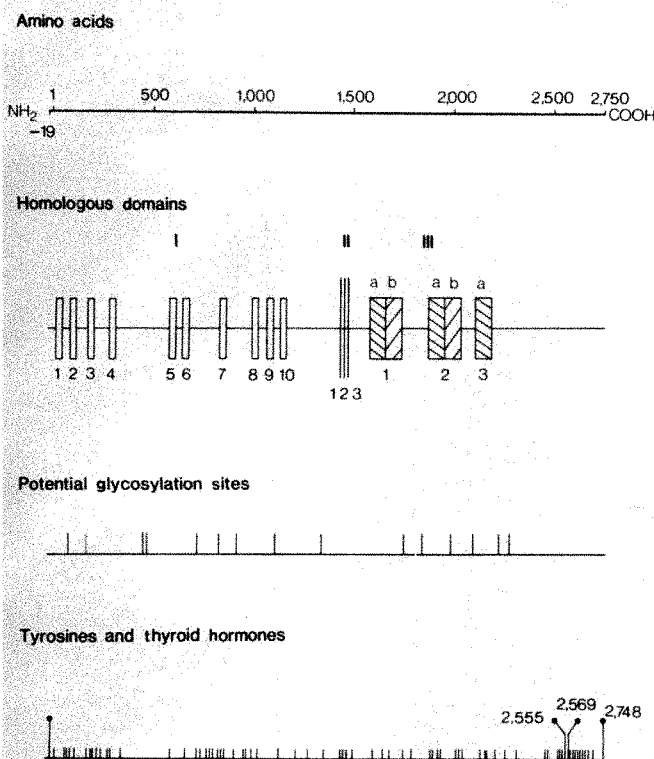


Fig. 4 Schematic representation of the thyroglobulin precursor. For clarity, a space has been introduced between the homologous domains even when they are contiguous in the protein (see Fig. 2). The tyrosine and thyroid hormone residues are represented by short and longer bars, respectively.

The sequence organization of thyroglobulin (Fig. 4) is reminiscent of the structure of some other large polypeptides, including the epidermal growth factor (EGF) precursor¹⁴, EGF receptor¹⁵ and low-density lipoprotein receptor¹⁶. Although there exists no direct sequence homology between thyroglobulin and these proteins, they probably share an analogous evolutionary history and may have similar three-dimensional structures.

Four tryptic peptides containing thyroid hormones have been isolated from thyroglobulin of various species and sequenced¹⁷⁻¹⁹. A systematic search for homologous peptides in our sequence allowed the identification and precise localization of all four hormonogenic tyrosines (Figs 3, 4). Two of these map at subterminal positions (residues 5 and 2,748); they correspond to sites involved preferentially in the synthesis of T₄ and T₃, respectively^{17,19}. The other two sites are closely linked around position 2,560. The amino-terminal domain is adjacent to the first, type I, repeat and might even share sequence characteristics with it⁵. In contrast, the three other hormonogenic tyrosines map in the non-repeated carboxy-terminal segment (Fig. 4). Comparison of the sequences surrounding the hormonogenic residues reveals no clear homology except for the presence of at least one charged amino acid (Fig. 3) and some clustering of tyrosines (Fig. 4)—the significance of this observation awaits the identification of the donor tyrosine involved in the coupling reaction.

Compilation of the sequences of the hormonogenic segments presently available shows a remarkable conservation of the amino-terminal domain (Fig. 3). It has been reported that as much as 50% of the thyroxine in mature thyroglobulin may originate from this domain²⁰. Although less information is available for the other hormonogenic domains, they do not display the same degree of sequence conservation (Fig. 3). Given these considerations, it is possible that the amino-terminal region of thyroglobulin has an important role in hormonogenesis. It is noteworthy, in this context, that a 10K amino-terminal peptide is cleaved from the protein during the hormonogenic process²¹.

The chemical or enzymatic reactions responsible for this phenomenon are unknown; it must be quite specific, however, as the carboxyl terminus of the 10K peptide could be unambiguously determined and was readily localized (Gln 80) in a partial protein sequence²².

Knowledge of the primary structure of thyroglobulin should provide the basis for studying the structure-function relationships of the hormonogenic process *in vitro*, in reconstructed synthetic systems, and should help to elucidate the molecular basis for genetic defects of thyroglobulin production²³.

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Ethylation interference and X-ray crystallography identify similar interactions between 434 repressor and operator

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In the crystal structure of a repressor-operator complex (the 434 repressor DNA-binding domain and its 14-base pair (bp) operator), Anderson *et al.*¹ elsewhere in this issue identify six positions of likely contact between repressor protein and phosphates of the DNA backbone. At each of these positions, electron densities of protein and DNA merge. Experiments presented here indicate that intact 434 repressor approaches these phosphates very closely when it is bound to DNA in solution. Specifically, when any one of these phosphates is ethylated², repressor cannot bind to the modified operator. We also identify another position where ethylation has

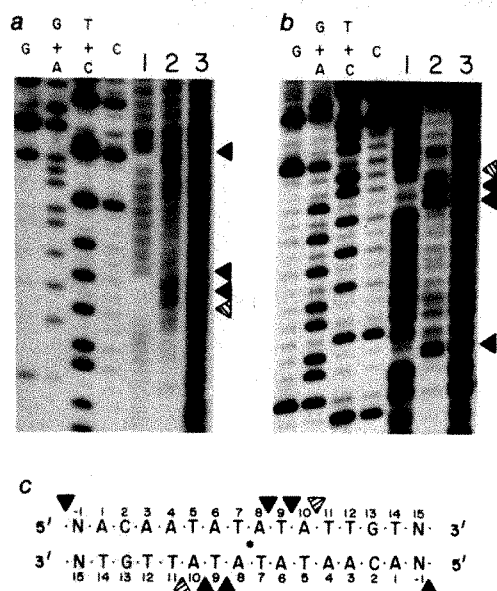


Fig. 1 Ethylation interference analysis of 434 repressor-operator interactions. *a*, Results for top strand; *b*, results for bottom strand. Lane 1, DNA bound by repressor; 2, DNA not bound by repressor; 3, ethylated unseparated control. In *a* and *b*, bands depleted in lane 1 and enriched in lane 2 relative to the control in lane 3 indicate ethylphosphates that interfere with repressor binding. *c*, Ethylphosphates that interfere with repressor binding aligned with the 434 operator sequence. Filled triangles, strong interference; hatched triangles, weaker interference.

Methods. A double-stranded consensus 2-fold-symmetric 434 operator of identical sequence to that used by Anderson *et al.*¹ with single-stranded *SalI* ends was generated by self-annealing of an oligonucleotide of sequence 5' TCGAACAATATATATTGT 3' (made on an Applied Biosystems Model 380A DNA synthesizer). This fragment was treated with T4 kinase (BRL) and ATP, then cloned using standard methods⁷ into the unique *SalI* site within the *lacZ* gene of pEMBL8- (ref. 8) to generate pFB37. Because insertion of the oligonucleotide destroys the *SalI* site, vectors without the insert in the ligation product could be linearized by digestion with *SalI* and hence eliminated during transformation. The 18-bp insertion preserves the *lacZ* translational reading frame by inserting 6 codons, none of which are translational stops. Plasmids with cloned operators could therefore be identified by transforming *Escherichia coli* strain JM101 (ref. 9) (phenotypically LacZ-), plating on X-Gal¹⁰ and picking blue Lac Z+ colonies. Plasmids containing single operators were identified by DNA sequencing¹¹ and cleaved at the unique *EcoRI* site. Cleaved plasmids were 3'-labelled (top strand) with DNA polymerase I large fragment (NEB) and [α -³²P]dATP (NEN), or 5'-labelled (bottom strand) by successive treatments with bacterial alkaline phosphatase (Boehringer) and T4 kinase and [γ -³²P]ATP (ICN; reactions as in ref. 7). After cleavage with *HindIII*, the labelled operator-containing fragment was isolated by electrophoresis on a 5% polyacrylamide gel, excision of the desired band and electroelution in 0.2× TAE buffer⁷. After filtration (Milex 0.45- μ m HA filters), phenol extraction, ether extraction and ethanol precipitation, DNA was ethylated with ethylnitrosourea (Sigma) as described². Ethylnitrosourea-treated DNA ($\sim 5 \times 10^{-8}$ M) was incubated at 4°C with 2×10^{-7} M 434 repressor (isolated as described previously¹²) in 50 mM KCl, 2 mM MgCl₂, 10 mM Tris pH 7.4, 0.5 mM EDTA, 1 mM dithiothreitol, 50 μ g ml⁻¹ bovine serum albumin and 5% glycerol. Repressor-operator complexes were separated from unbound DNA that contained ethylations interfering with repressor binding by polyacrylamide gel electrophoresis³⁻⁵. Bound and unbound fractions were excised from the gel and purified as described above. Ethylated DNA fragments were cleaved at phosphotriesters by heating in alkali², prepared for electrophoresis as described³ and analysed on a 10% sequencing gel. Ethylation cleavage products were aligned with the sequencing ladder by correcting down to the nearest base (see ref. 2 for discussion). Alignment of a 5'-labelled sequencing band with a 5'-labelled ethylation cleavage product identifies the phosphate 5' to that base; alignment of a 3'-labelled sequencing band with a 3'-labelled ethylation cleavage product identifies the phosphate 3' to that base.

a significant but less dramatic effect on repressor binding, and note that in the structure, repressor closely approaches this phosphate. Our results strongly support the idea that the interactions between protein and the DNA phosphate backbone in the crystallized complex¹ are the same as those made by intact repressor to operator DNA in solution. In addition, our results suggest that DNA is slightly bent by repressor binding.

Phosphates of the 434 operator which, when ethylated, interfere with 434 repressor binding were identified by the method of Siebenlist and Gilbert² (modified as described by Hendrickson and Schleif³, see Fig. 1 legend). Briefly, the 2-fold-symmetric consensus 434 operator used by Anderson *et al.*¹ was cloned on a plasmid, end-labelled and ethylated such that each DNA molecule had an average of no more than one ethylphosphate. Purified ethylated fragment was incubated with excess 434 repressor, then operators bound to repressor were separated from the unbound operators by electrophoresis³⁻⁵. The unbound DNA molecules contain ethyl groups that prevent binding. Bound and unbound DNA fragments were isolated, cleaved at sites of ethylation and examined by electrophoresis on a polyacrylamide sequencing gel. Each band on the gel is generated by cleavage at a specific ethylphosphate. Bands enriched in the unbound fraction and depleted in the bound fraction indicate phosphates at which ethylation inhibits repressor binding.

Ethylation at any of three phosphates found 5' to bases -1, 9, and 10 on each strand prevents binding at the repressor concentration used here (compare lanes 1 and 2, Fig. 1*a, b*). Anderson *et al.*¹ show that these phosphates lie near residues Asn 16 and Gln 17 at the N-terminus of helix α 2 (phosphate -1); and Lys 40, Arg 41 and Arg 43 (phosphates 9 and 10). Ethylation of a fourth phosphate, 5' to base 11, has a significant but weaker effect on binding (hatched triangles in Fig. 1). In our interpretation of the structure, this phosphate is positioned within reasonable hydrogen-bonding distance of the γ -O of Ser 30. Thus, there is excellent agreement between the results of these chemical probe experiments and the co-crystal structure. The four phosphate contacts are symmetrically disposed about the centre of the operator (Fig. 1*c*) on one face of the B-DNA helix (Fig. 2).

Ethylation of a DNA phosphate removes the negative charge and adds a group with a van der Waals radius of ~ 4.5 Å. Both steric and charged-group effects can therefore contribute to its influence on binding. Model building with straight DNA shows that with the relationship of DNA and protein seen in the crystal, the side chains of Asn 16 and Gln 17, the only residues near



Fig. 2 Phosphates of the 434 operator at which ethylation reduces affinity for 434 repressor. Positions of strong ethylation interference are symbolized by filled circles and weaker interferences by hatched circles on a B-DNA helix. The operator centre of symmetry is indicated with a small filled circle.

the -1 phosphate, are too far from the DNA backbone for hydrogen-bond formation (Fig. 3a). If the DNA is bent to a radius of curvature of ~ 100 Å, then a hydrogen bond to the -1 phosphate can readily be made (Fig. 3b). Other small deformations could have a similar effect. An additional contribution to binding might come from favourable interaction between the partial positive charge on the N-terminus of helix $\alpha 2$ and the negative charge on the phosphate⁶. Based on the observed significance of the interaction at the -1 phosphate, we propose an explanation for some aspects of the crystal structure. In the co-crystal, 14-bp operators, each bound by a dimer of repressor DNA-binding domain, pack end-to-end to form rods. Each

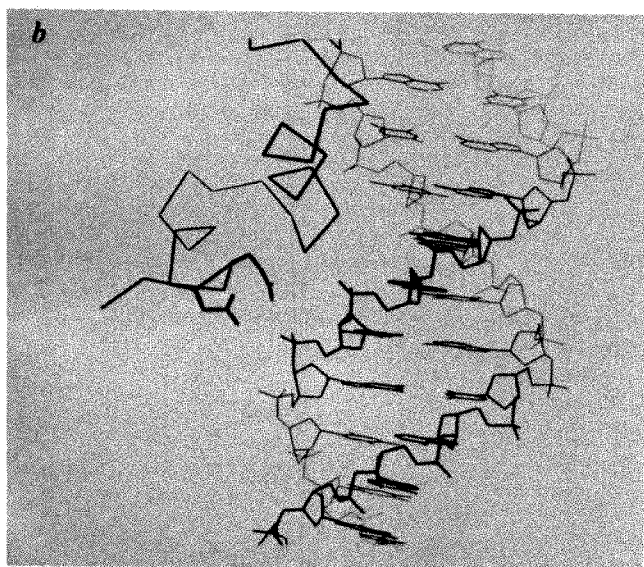
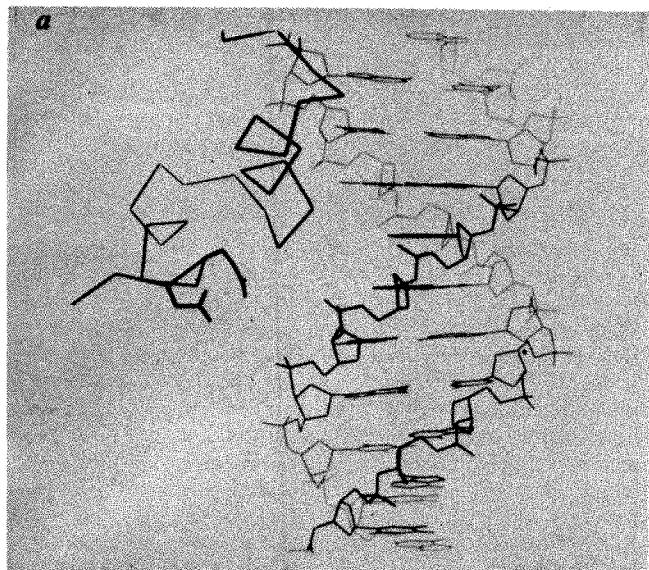


Fig. 3 Interaction of Asn 16 and Gln 17 with straight and bent DNA showing the α -carbon backbone of λ -repressor residues 30–60 (ref. 13), positioned as described by Anderson *et al.*¹, with the side chains of Asn 16 and Gln 17 of 434 repressor extending toward the DNA. In *a*, straight B-DNA was constructed by extending the double helix of the model DNA that best fits the electron density of one 14-bp operator¹. The amino nitrogens of the Asn 16 and Gln 17 side chains are both >5 Å from the oxygens of the -1 phosphate, which is too far for hydrogen-bond formation. In *b*, B-DNA with a slight right-handed supertwist, generated as described by Anderson *et al.*¹, was positioned so that 14 bp of the modelled DNA gave the best fit to the electron density of one 14-bp operator. The side-chain amino groups of Asn 16 and Gln 17 are separated from the phosphate oxygens by ~ 3 Å, a typical hydrogen-bond length.

14mer is tilted slightly and displaced laterally with respect to the next, so that the 3' terminal base on each strand does not stack on the adjacent operator. A repressor monomer bound to a given 14mer reaches over to an adjacent 14mer to make the -1 phosphate contact, and the DNA structure deviates from regular B-like conformation in this region. We imagine that the tilting and displacement of the 14mers, and possibly the local departure of the DNA backbone from canonical B-form, are consequences of crystallization using discrete fragments rather than continuous DNA. We believe that these effects occur in order to accommodate the -1 phosphate interaction, which in continuous DNA seems to require a gentle bend.

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Photodriven transmembrane charge separation and electron transfer by a carotenoporphyrin–quinone triad

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Photochemical transmembrane electron transfer processes are an integral part of natural photosynthetic solar energy conversion and are also central to the design of biomimetic energy conversion schemes^{1–6}. Here we report the synthesis and membrane-associated photoelectrochemical properties of carotenoporphyrin–quinone triad (I), a compound containing a photochemically active porphyrin and electron donor and acceptor moieties, and with the molecular architecture necessary to span a phospholipid bilayer. On excitation of compound I by visible light, charge is separated across a planar phospholipid bilayer membrane (BLM) in an intramolecular step; in the presence of suitable electron donor and acceptor species in the aqueous phases, a steady-state photocurrent is observed in an external circuit bridging the BLM. Artificial membranes containing I thus mimic key features of the photodriven transmembrane electron transfer processes characteristic of photosynthetic organisms.

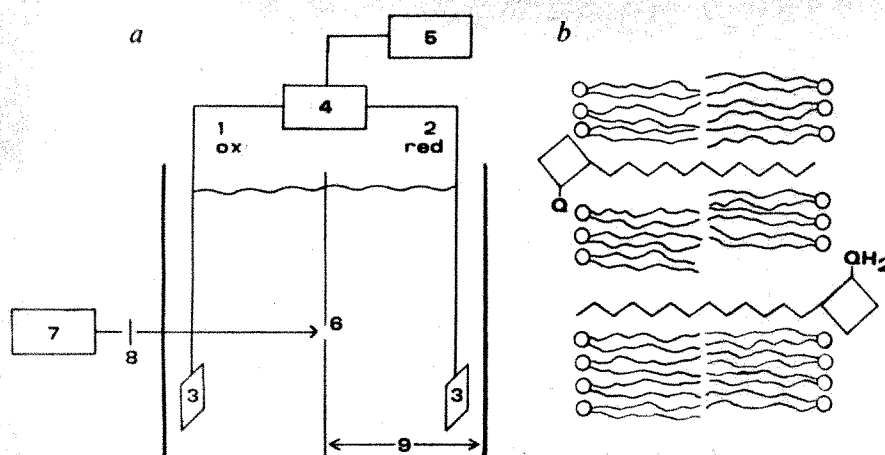


Fig. 1 *a*, Schematic diagram of a BLM cell for photoelectrical measurements: 1, side 1 containing oxidant ($\text{Fe}(\text{CN})_6\text{K}_3$), phosphate buffer and NaCl; 2, side 2 containing reductant (sodium ascorbate), phosphate buffer and NaCl; 3, Ag/AgCl electrode; 4, Keithley model 427 current-to-voltage converter; 5, Tektronix digital storage oscilloscope; 6, 1-mm-diameter orifice in thin Teflon divider; 7, laser; 8, shutter; 9, cell machined from block of Teflon. *b*, Schematic diagram showing the postulated arrangement of triads in the phospholipid bilayer. Note that triad I (labelled Q) is shown at the aqueous interface on side 1 and triad II (QH_2) at the interface on side 2.

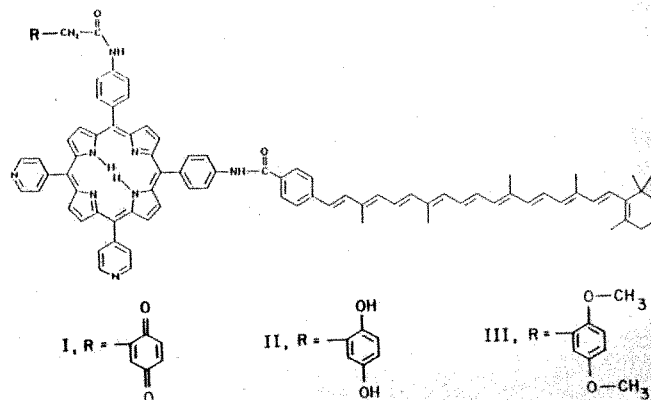
Photodriven charge separation in single-phase solution in compounds similar to I has been shown to be a two-step process involving electron transfer from the photoexcited porphyrin singlet state to the attached quinone, followed by electron transfer from the carotenoid to the porphyrin radical cation⁷⁻¹⁰. The resulting species, $\text{C}^{++}-\text{P}-\text{Q}^{--}$, is formed within ~ 100 ps of excitation and has a solvent-dependent lifetime on the micro-second timescale.

Triad I and its related forms II and III (see above) were synthesized by routes similar to those used previously^{7,11}. Their structures were verified using 500-MHz ^1H -NMR spectroscopy¹².

The photoelectrochemical experiments were carried out in the apparatus described previously¹³ (see Fig. 1*a*). The triad was dissolved in the appropriate membrane-forming solution (see Fig. 2 legend) and a small drop applied to the orifice in the Teflon divider. The phospholipid solution gradually thinned and formed a bilayer, as detected by coulometric measurements¹³. The current flowing through the cell, and therefore through the BLM, was detected as a function of time and displayed on a digital oscilloscope. In a typical experiment with triad I in the membrane, 1×10^{-2} M $\text{Fe}(\text{CN})_6\text{K}_3$ was dissolved in the aqueous phase on side 1 and 1×10^{-2} M ascorbic acid was placed on side 2. In the dark, no significant current was observed. However, irradiation of the BLM with 600-nm light from the laser produced an immediate photocurrent (Fig. 2). Typically (Fig. 2*a*), the photocurrent consisted of a transient component that rose with the opening time of the shutter (< 1 ms) and quickly decayed to a steady-state value (typically 50–500 pA). The peak of the transient current and the steady-state value varied considerably between samples. Generally, there was a strong positive correlation between the extent of bilayer formation, as estimated from the coulometric measurements, and the magnitude of the photocurrent. Moreover, on average, photocurrents obtained when the membrane-forming solution was prepared with hexadecane were larger than those when decane was used. This observation is consistent with the fact that solvents comprising hydrocarbon of longer chain length yield thinner membranes¹⁴. Figure 2*b* shows a photocurrent in which the transient component was not observed; such signals were less common than the first type, but occurred more frequently when the aqueous phases were at low pH and when the membranes were more than 10 min old and very thin (that is, when hexadecane was used as the solvent).

The currents discussed above are clearly the result of photo-induced electron transport across the phospholipid bilayer. We propose the following explanation for the results. The triad is a rod-like, amphipathic molecule that would be expected to lie in the bilayer as shown in Fig. 1*b*. The hydrocarbon-like carotenoid moiety (which extends ~ 39 Å from the porphyrin centre) should be well anchored in the low-dielectric membrane interior and should be aligned to some extent with the fatty-acid chains. The polar dipyrrolic porphyrin moiety, on the other hand, should lie in the higher-dielectric region near the head groups.

This is particularly the case at pH 3, where one would assume that the dipyrrolic porphyrin was positively charged. Because of its short connecting link to the porphyrin, the quinone must also reside near the membrane surface. In addition, the membrane is asymmetric by virtue of the fact that even though the triad is added to the membrane-forming solution in the hydroquinone form II (because it is more soluble), the addition of $\text{Fe}(\text{CN})_6\text{K}_3$ to the solution on side 1 (Fig. 1*a*) converts the molecules having their hydroquinone moieties at the side 1 aqueous interface to the quinone form. Those molecules having their hydroquinone moieties at the side 2 interface remain in that oxidation state. (This interfacial oxidation was demonstrated in bulk two-phase mixtures of the membrane-forming solution and aqueous ferricyanide, using chromatographic detection. No detectable decomposition of the pigments in such bulk mixtures was noted even after several hours of contact.)



Irradiation of a triad quinone I in such a bilayer is postulated to produce the $\text{C}-\text{P}^{++}-\text{Q}^{--}$ charge-separated state, and ultimately the $\text{C}^{++}-\text{P}-\text{Q}^{--}$ state, as observed in similar monophasic systems⁷⁻¹⁰; this would lead to a quinone radical anion near the aqueous interface on side 1, and a carotenoid radical cation in the interior of the bilayer. The quinone radical anion then donates an electron to ferricyanide to regenerate the neutral quinone, and the carotenoid radical cation, which can nearly span the bilayer, can accept an electron from the ascorbate on side 2 to regenerate the neutral carotenoid. The net result is the vectorial transfer of an electron from side 2 to side 1 and the regeneration of the triad photocatalyst. The charge separation across the membrane is countered by electron flow through the electrodes and detected as current in the external circuit.

There are several lines of evidence supporting the above interpretation of the experimental results. First, laser-flash photolysis of phosphatidylserine (or ethanolamine) liposomes containing compound I with 590-nm light resulted in a transient species having a maximum wavelength (λ_{max}) of 970 nm; the spectrum of this transient confirmed that it was indeed the

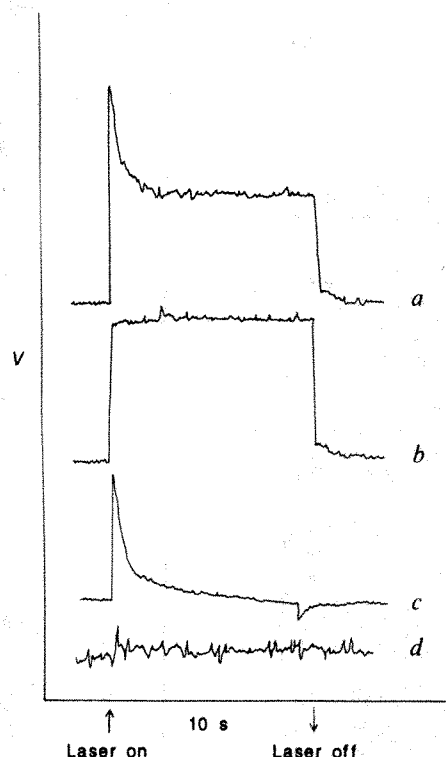


Fig. 2 Transmembrane photocurrents observed on irradiation of triads in BLM systems. *a*, pH 6.5, peak current 200 pA, 1×10^{-2} M ascorbate in side 2 and 1×10^{-2} M $\text{Fe}(\text{CN})_6\text{K}_3$ in side 1; *b*, pH 2.7, current 150 pA, same redox couples as in *a*; *c*, pH 5, peak current 29 pA, oxidizing couple as in *a*, no reductant in side 2, hexadecane as the solvent; *d*, triad III, noise peaks are ~ 5 pA, conditions as in *a*. The membrane-forming solutions used phosphatidylserine or phosphatidylethanolamine (3 mg), decane (100 μl) and ~ 0.05 mg of triad. In each case the membrane showed substantial thinning, as measured by coulometric response, greater than $5 \text{ G}\Omega$ d.c. resistance, and a dark current of < 5 pA. Each aqueous phase contained 0.1 M NaCl, 1×10^{-3} M phosphate buffer and a freshly prepared Ag/AgCl electrode. The sign of the observed currents is consistent with a negative charge carrier flowing from side 2 to side 1 through the bilayer. In these experiments there was no pH gradient (ΔpH) across the membrane. For *a*, *b* and *c*, triad II was used in the membrane-forming solution as described in the text.

carotenoid radical cation. Thus, the postulated charge separation does occur in the phospholipid bilayer. Furthermore, when the electron donor (ascorbate) was omitted from side 2, large transient photocurrents were observed on illumination, but a steady-state photocurrent was never detected (Fig. 2c). Similarly, no steady-state current was observed in the absence of ferricyanide. In addition, no transmembrane electrical response was detected for triad III in the conditions used for I

(Fig. 2d). In III, the hydroquinone moiety is protected as the dimethyl ether, and formation of the intramolecular charge-separated state is precluded. These three control experiments confirm the involvement of the transmembrane charge-separated state of triad I and the aqueous-phase redox couples in the observed steady-state photocurrents.

A final piece of evidence concerns the turnover number of the triad in the BLM. If the proposed scheme is indeed correct, then each triad I molecule should be capable of transporting many electrons across the bilayer. At the excitation wavelength of 600 nm, the porphyrin absorption cross-section is $\sim 1.7 \times 10^{-17} \text{ cm}^2$ per molecule and the laser intensity was $\sim 3 \times 10^{18}$ quanta per s cm^2 , yielding ~ 50 excitations per s molecule. From the composition of the membrane-forming solution, there are an estimated 10^{10} molecules per BLM and therefore 5×10^{11} excitations per s in the BLM. At a quantum yield of $\sim 10\%$ (refs 7–10), this corresponds to a photocurrent of $\sim 4 \text{ nA}$, a value recorded in some of the BLM experiments. Although very approximate, this result is certainly consistent with a steady-state process having a high turnover number and capable of operating for many seconds.

Note that this example of transmembrane electron transfer is essentially the light-driven catalysis of the thermodynamically spontaneous oxidation of ascorbate by ferricyanide ion. None of the intramolecular redox potential of the charge-separated state $\text{C}^{++}-\text{P}-\text{Q}^{\cdot-}$ is conserved as transmembrane chemical potential. However, photocurrents opposing the applied electrical potential were observed even when side 1 was 100 mV negative to side 2. The judicious choice of redox couples and a properly asymmetrized membrane should ultimately make it possible to achieve net energy conservation in this system.

In photosynthetic membranes, carotenoids prevent the sensitization of oxygen by chlorophyll triplet-state species and thereby perform a photoprotective function^{15,16}. These carotenoporphyrin triads have sufficient electronic coupling between the carotene and porphyrin moieties¹¹, via the amide linkage, to mediate extremely rapid triplet-triplet energy transfer ($\text{C}-\text{P}-\text{Q} \rightarrow {}^3\text{C}-\text{P}-\text{Q}$)⁹. Therefore, even though excitation of the hydroquinone triads results in a high yield of porphyrin triplet, energy transfer to the attached carotenoid occurs on the nanosecond timescale and precludes singlet oxygen formation. These artificial membranes are thus photoprotected in precisely the same way as native photosynthetic membranes.

The photodriven charge separation by carotenoporphyrin-quinone triads across artificial membranes, as demonstrated here, serves as a paradigm for many transmembrane electrochemical events in energy-transducing biological membranes, and will allow detailed investigations of photodriven charge separation across both chemical and electrical potentials, which should in turn lead to a better understanding of photosynthetic energy conversion.

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Hydrogen bonding in enzymatic catalysis analysed by protein engineering

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Two historic publications have suggested that enzymes can utilize the binding energy with their substrates to increase the rate of chemical catalysis—a concept termed strain^{1,2}. Specifically, the structure of an enzyme was described as complementary to the structure of the transition state of its substrate rather than to that of the unreacted substrate, so that the substrate would tend to be distorted on binding to the enzyme. The current understanding of strain does not require such distortion. Instead, it is thought that there are groups on the enzyme which interact better with the transition state than with the unaltered substrate, an arrangement known as differential binding of substrates and transition states³. The improvement in binding energy caused by going from substrate to transition state lowers the activation energy of the reaction and so increases the catalytic rate. Here we report direct evidence in support of this notion. Rapid reaction kinetics on mutants of a tyrosyl-transfer RNA synthetase produced by protein engineering show that certain hydrogen-bonding side chains some distance from the reacting bonds of the substrate bind to ATP preferentially in the transition state of the reaction rather than in the unreacted state. In this way, the binding energies of the interactions are used primarily to lower the apparent activation energy of the chemical steps rather than to enhance binding.

The hypothesis of better binding in the transition state has been tested using substrates and inhibitors with varying structures. Two lines of supporting evidence have been adduced from classical enzyme kinetics³. First, transition-state analogues, which usually mimic the changes in geometry about the bonds that are being broken and made in the reaction, often bind between 10² and 10⁴ times more strongly than the original substrates. Second, experiments on proteases with broad specificity show that additional groups on a substrate can lead to an increase in k_{cat} rather than stronger binding. These experiments suggest that groups on the substrate that are far from the seat of reaction can alter the catalytic rate. Many of the substrates

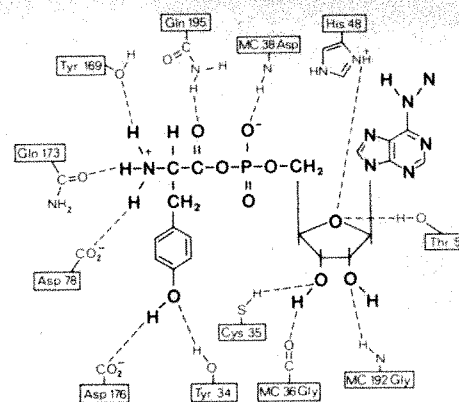
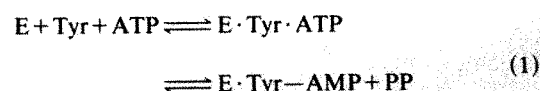


Fig. 1 Side chains of the tyrosyl-tRNA synthetase that form hydrogen bonds with tyrosyl adenylate (courtesy of D.M. Blow and P. Brick).

used in these studies, however, differ greatly from each other or from the specific substrates, and the results may arise simply from the nonproductive binding of the smaller substrates³.

The advent of protein engineering allows the idea of differential binding of transition states and substrates to be tested directly by varying the structure of the enzyme. An amino-acid side chain that interacts with the substrate can be altered to remove the interaction. If the side chain binds equally well with the substrate in both the ground and transition states, then removal should raise the dissociation constant K_S and not affect k_{cat} . Conversely, if the side chain binds the substrate only in the transition state, then removal should leave K_S unaltered and just lower the value of k_{cat} . Importantly, just one interaction out of many with the substrate may be changed at a time so that artefacts associated with a change of mode of binding of the substrate will be minimized. The contributions of individual interactions to differential binding energies are not known and this approach enables them to be quantified.

Genes of mutant tyrosyl-tRNA synthetases (from *Bacillus stearothermophilus*), which have had the relevant hydrogen-bonding groups removed, have been generated for use in experiments to determine the role of hydrogen bonding in biological specificity⁴. Values of k_{cat} and K_M for tyrosine and ATP for the activation reaction (equation (1)) were measured by pyrophosphate



exchange. Unfortunately, because of the complex kinetics, the values of k_{cat} and K_M for pyrophosphate exchange are composite

Table 1 Activation of tyrosine by tyrosyl-tRNA synthetases

Enzyme	k_3 (s ⁻¹)	K'_S (ATP) (mM)	K_S (Tyr) (μM)
Wild type	38	4.7	12
Δ(321-419)*	34	5.2	12
Tyrosine binding-site mutants			
Tyr → Phe 34	35	4.4	29
Tyr → Phe 169*	35	4.6	1,320
ATP binding-site mutants			
Cys → Ser 35	4.7	4.8	8
Cys → Gly 35	4.0	4.5	11
His → Gly 48	9.9	9.9	23‡
Thr → Ala 51	75	4.7	12

Experiments performed at 25 °C at pH 7.78 (144 mM Tris-HCl) in the presence of 10 mM free MgCl₂. k_3 , Rate constant for the formation of E.Tyr-AMP obtained by stopped-flow fluorimetry⁸, with standard error ±5%. K'_S (ATP) dissociation constant of ATP from the E.Tyr.ATP complex, determined as for k_3 ; K_S (Tyr), dissociation constant of Tyr from the E.Tyr complex obtained from equilibrium dialysis⁸.

* Truncated enzyme lacking the tRNA binding domain (residues 320-419)⁹.

† Mutation on truncated enzyme with the reference enzyme being the truncated wild-type enzyme.

‡ This value is abnormally high and may be caused by a loss of an electrostatic interaction with His 48.

Table 2 Apparent binding energies of side chains to substrates and to transition states

Side chain	Binding energy with substrate* (kcal mol ⁻¹)	Binding energy with transition state† (kcal mol ⁻¹)
Tyrosine binding site		
Tyr 34 —OH	-0.52	-0.57
Tyr 169 —OH	-2.58	-2.58
ATP binding site		
Cys 35 —SH	-0.03	-1.21
His 48 —imidazole	-0.44	-1.24
Thr 51 —OH	0.00	+0.40

* Calculated from $\Delta G = -RT \ln K_{mut}/K_{wt}$, where K is the relevant dissociation constant of the substrate and mut and wt refer to mutant and wild-type enzymes respectively.

† Calculated from $\Delta G = RT \ln (k_3/K)_{mut}/(k_3/K)_{wt}$.

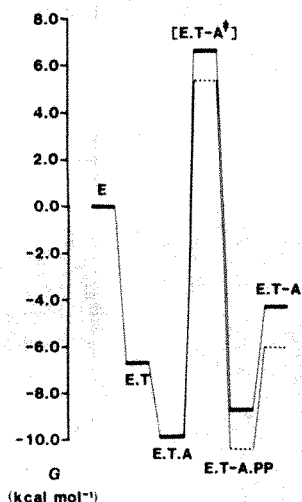
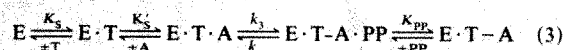
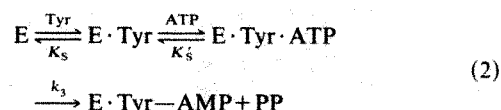


Fig. 2 Gibbs free-energy profiles for the formation of tyrosyl adenylate (E.T-A) by wild-type enzyme (---) and the mutant Cys→Gly 35 (—) (equation (3)). The rate constants for the formation of E.T-A and the transition state E.T-A* are taken from Table 1.



The reverse rate constant k_{-3} (16 s^{-1} for wild type, 33 s^{-1} for mutant) and dissociation constant for pyrophosphate, K_{PP} (0.61 mM for wild type, 0.63 mM for mutant) were measured by stopped-flow fluorimetry⁷. Free energies were calculated from the standard equations using a standard state of 1 M for tyrosine (T), ATP (A) and pyrophosphate.

values containing rate and equilibrium constants for the pyrophosphorolysis reaction. These data thus gave no conclusive evidence on the interconversion of binding and chemical activation energies. Values for k_3 and the dissociation constants (K_S and K'_S) in equation (2) can, however, be measured



directly by stopped-flow fluorimetry⁵ and equilibrium dialysis (Table 1). From these values, free-energy contributions to binding and catalysis for individual groups can be calculated using the standard equations³ (Table 2).

The residues that were mutated are all in the binding site of the enzyme, but are not directly involved in bond making and breaking (Fig. 1). Removal of side chains that bind to tyrosine (Tyr→Phe 34 and Tyr→Phe 169) does not significantly alter k_3 but increases the dissociation constant of tyrosine from the $E \cdot \text{Tyr} \cdot \text{ATP}$ complex (Table 1). The hydroxyl moieties of Tyr 34 and Tyr 169, therefore, can be assumed to interact equally well with tyrosine in its unreacted form and in the transition state of the reaction. The binding energies of the -OH groups of Tyr 34 and Tyr 169 with the substrate and transition state are readily calculated³ and are seen to be nearly identical (Table 2).

In contrast, removal of side chains that interact with ATP causes significant changes in k_3 but, apart from His→Gly 48, hardly affects the value of K'_S for ATP. Mutation of Cys 35 to Gly 35 or Ser 35 leads to a 10-fold lowering of k_3 with no significant change in K'_S . Changing His→Gly 48 results in a mixture of effects, including a fourfold lowering of k_3 and a twofold increase in K'_S . Mutation of Thr→Ala 51 leads to a twofold increase in k_3 with no discernible effect on K'_S for ATP. This result is entirely consistent with the earlier proposal from structural observations and steady-state kinetic measurements that the bond between Thr 51 and the ribose ring oxygen is long and weak and contributes no binding energy^{5,6}. As the value of k_3 is increased on deletion of this bond, it must be even longer and weaker in the transition state. (The presence of an alanine at position 51 gives a more active enzyme, and it is intriguing

that the tyrosyl-tRNA synthetase from *Bacillus caldotenax*, which is 99% homologous with that from *Bacillus stearothermophilus*, does have alanine at position 51; ref. 7).

Thus, the side chains of residues 35, 48 and 51 probably have different interaction energies with ATP depending on whether it is in its unreacted form or its transition-state structure. The differences could arise from two effects, or a mixture of the two: (1) an unfavourable interaction in the enzyme-substrate complex that is relieved on formation of the transition state (substrate destabilization); and (2) an interaction that is not properly made in the enzyme-substrate but is realized in the enzyme-transition state complex (transition-state stabilization). We have shown by construction of the full free-energy profile for the formation of tyrosyl adenylate (Fig. 2) that the side chain of Cys 35 does not function purely by substrate destabilization. Removal of the side chain of Cys 35 (Cys→Gly 35) lowers the affinity of the enzyme for tyrosyl adenylate by the full amount expected for the loss of a hydrogen bond⁴, showing that it makes favourable interactions with the adenosine moiety of the products as well as with the transition state.

It seems unlikely from model building that the mutation of residues in this study will cause any structural artefact. Further, the double-mutant test has been applied to residues 35 and 48 and no change of structure was detected⁶.

We have concluded, therefore, that the interactions of important side chains of the enzyme with tyrosine remain unaltered but that those with ATP are optimized as the enzyme-substrate complex reaches the transition state. Thus we have shown that the binding energy of the enzyme and substrate can be used to enhance the catalytic rate and we have quantified its importance.

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Corrigenda

The nucleotide sequences of *copia* and *copia*-related RNA in *Drosophila* virus-like particles

T. Emori, T. Shiba, S. Kanaya, S. Inouye, S. Yuki & K. Saigo
Nature **315**, 773-776 (1985)

ALL U3s and U5s on page 776, lines 16-17 and in Fig. 2 should be U5s and U3s respectively.

Analytical solution for the effect of increasing CO₂ on global mean temperature

T. M. L. Wigley & M. E. Schlesinger
Nature **315**, 649-652 (1985)

IN Fig. 1 legend, the lower part of the figure should be that for $\kappa = 3 \text{ cm}^2 \text{ s}^{-1}$ and the upper part for $\kappa = 1 \text{ cm}^2 \text{ s}^{-1}$ and not vice versa. In the acknowledgements the NSF/DOE grant number should be ATM 8205-992.

Erratum

X-linkage of steroid sulphatase in the mouse is evidence for a functional Y-linked allele

E. Keitges, M. Rivest, M. Siniscalco & S. M. Gartler
Nature **315**, 226-227 (1985)

The first line of the first column on p. 227 was printed in the wrong position and should be read as the last line of the first column of that page.

Aromaticity of soil fulvic acid

FARMER and Pisaniello¹ claim that: (1) aliphatic and aromatic oxidation products of the podzol Bh fulvic acid, especially after premethylation with diazomethane, are mainly artefacts or contaminants; and (2) the formation of these oxidation products cannot be reconciled with the ¹³C-NMR spectrum of the fulvic acid.

I have the following comments: (1) Benzenecarboxylic, phenolic and aliphatic carboxylic acids are not only the major oxidation products of the alkaline KMnO₄ oxidation of unmethylated and methylated fulvic acid², but also result from the oxidation of unmethylated fulvic acid with alkaline CuO (ref. 3) and alkaline H₂O₂ (ref. 4) as well as HNO₃ (ref. 5) and CH₃CO₂OH (ref. 6). Thus, diazomethane has nothing to do with the types of products resulting from the oxidation of the fulvic acid.

(2) Both solid-state⁷ and liquid-state (refs 8, 9) ¹³C-NMR spectra of fulvic acid show signals near 20 and 30 p.p.m., indicative of the presence of terminal CH₃ and (CH₂)_n in alkyl chains. Other resonances are detected between 50 and 85 p.p.m., arising from amino acids and carbohydrates. Major signals near 130 p.p.m. indicate the presence of aromatic carbons not substituted by O or N. But in the liquid-state ¹³C-NMR spectrum of the methylated fulvic acid⁸, now soluble in CDCl₃, the broad band near 130 p.p.m. is resolved into four sharp signals, clearly indicating improved resolution. The comments of Farmer and Pisaniello on this point are irrelevant because they erroneously assumed that this spectrum was run in base instead of CDCl₃. Other strong signals in the ¹³C-NMR spectrum of fulvic acid appear near 180 p.p.m., due to carbon in CO₂H groups. While some humic substances show signals between 150 and 160 p.p.m., others do not. It is possible that because of substitution effects, the chemical shift due to phenolic carbon is moved downfield so that signals arising from phenolic carbon and carbon of CO₂H groups overlap. It is noteworthy that after methylation of the fulvic acid, the presence of phenolic carbon is indicated by a sharp signal at 56.3 p.p.m. Thus, contrary to the claims of Farmer and Pisaniello¹, the structural information on fulvic acid provided by ¹³C-NMR agrees with that indicated by chemical degradation.

Dialkyl phthalates are the only plasticizers that have so far been detected in oxidation products of fulvic acid². The oxidation of dialkyl phthalates would be expected to yield benzenedicarboxylic acids. But how can dialkyl phthalates be oxidized to benzenetri-, tetra-, penta- and hexacarboxylic acids and phenolic acids?

Therefore, it is highly unlikely that significant amounts of oxidation products arise from contaminants. Another important indicator of aromatic fulvic acid components is the relatively high content of free radicals⁴ that have the spectroscopic characteristics of semi-quinones. I conclude from the above that aromatic structures are significant constituents of fulvic acid. The question of whether or to what extent hydroxyl-substituted unsaturated aliphatic carboxylic acids are present in fulvic acid, awaits the isolation of significant amounts of such structures, which, so far, nobody has been able to do.

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FARMER REPLIES—Preston and Schnitzer¹ suggested that the four sharp aromatic carbon signals that they observed in the ¹³C-NMR spectrum of methylated fulvic acid might arise from benzene polycarboxylic esters, and supposed that the parent acids would not be detectable in ¹³C-NMR spectra of fulvic acid dissolved in alkali. We found² that such acids were readily detectable as sharp signals when added to an NaOD solution of fulvic acid: our comments are therefore entirely relevant, as they negate Preston and Schnitzer's hypothesis¹, and establish, conclusively, that simple benzene polycarboxylic acids are not even a minor component of fulvic acid. The four sharp signals observed in the methylated fulvic acid can arise from little more than four different types of benzenoid carbon. They are therefore unlikely to be associated with benzenoid carbons of the diverse types indicated by the multiple aromatic degradation products of fulvic acid, reported by Schnitzer and co-workers. The four bands are more likely to arise from a contaminant.

The references cited above by Schnitzer illustrate that reports of high yields of benzene and hydroxybenzene polycarboxylic acids in the oxidation products of humic and fulvic acids originate almost entirely from his laboratories. Consider-

ing, for example, only the non-phenolic benzene polycarboxylic acids, Schnitzer and co-workers have reported yields mostly in the range 6-14% of the starting materials. In contrast, Ogner³ and Spitteler⁴ obtained yields of only 0.12-0.38% of these acids. In a careful quantitative study using permanganate oxidation, H. A. Anderson of this institute (personal communication) has obtained a yield of 1.63% from a podzol Bh fulvic acid (Contech), similar to the Armadale preparation intensively studied by Schnitzer and co-workers. This yield does not greatly exceed that given by polymaleic acid in the same conditions (0.8%).

Following the suggestion² that ¹³C-NMR spectra could be used to estimate the amounts of benzene and hydroxybenzene polycarboxylic acids in the unfractionated oxidation products of fulvic acids, we have applied this technique to the products of peracetic acid oxidation. The results showed clearly that benzene polycarboxylic acids cannot be present in the amounts reported by Schnitzer and Skinner⁵.

In the absence of confirmation from other laboratories, the high yields of aromatic degradation products reported by Schnitzer and co-workers can carry no weight. We can only speculate on the reasons for these anomalously high yields.

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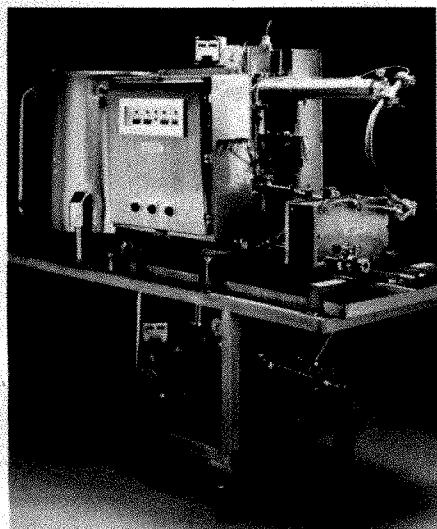
B-I-T-S

Laboratory fittings go Dutch

The new products covered here are from some of the manufacturers exhibiting at the 13th International Congress of Biochemistry in Amsterdam.

• Labsystems UK has recently introduced two new instruments, the FP9000 Analyser for automatic batch processing, random access and profile chemistries and the Haematology Analyser for coagulation work. Labsystem's other products include ELISA kits for *Toxoplasma gondii*, cytomegalovirus, rubella, *Chlamydia*, *Bordetella pertussis* and tetanus toxoid, plus the Fecatest, a human Hb specific ELISA for the detection of colorectal carcinoma.

Reader Service No. 100.



Prostak features a 'unique' integrity-testing facility.

• Prostak cell harvesting systems, new from Millipore's Systems Division, are aimed at particularly difficult cell separations, such as mammalian cell isolation, and cellular material that is produced in a complex, viscous broth. Prostak systems offer simplicity and yield advantages over traditional separation techniques, such as rotary drum filtration and centrifugation. And it offers performance advantages over traditional membrane separation devices, including validatability and steamability. The Prostak line includes both microporous membrane and ultrafiltration membrane devices. The devices are incorporated into pilot-plant and production systems that include all necessary pumps, tanks and instrumentation. The basic Prostak device is completely self-contained, and has no O-rings or gaskets that leak hazardous biologicals. The open channel design of the device eliminates plugging and maintains maximum cell viability. Each Prostak device can be in-

These notes are based on information provided by the manufacturers. To obtain further details about these products use the reader service card bound inside the journal.

tegrity tested in the system before each run, a feature that the manufacturer claims is unique to Millipore systems. Reader Service No. 101.

• A new 24-page catalogue presents Amicon's line of columns for liquid chromatography. It comprises laboratory, pilot and large production-scale columns of acrylic, glass and stainless steel. The latter include high-pressure columns capable of operation at 3,000 p.s.i. The design of these columns provides a logical system for performance scale-up, with increasing column diameters in steps of approximately doubled cross-sectional area. Modular design concepts make column tubes of different materials but the same diameter interchangeable for many sizes, for most economical use of hardware components.

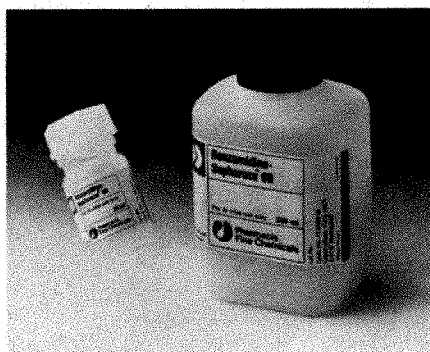
Reader Service No. 102.

• The second edition of J.T. Baker Chemicals' publication *Applications Scientist* is now available. The newsletter features articles on the complete HPLC separation of PTH amino acids and a rapid solid phase extraction of herbicides from aqueous and solid samples. Use the reader service card to obtain a free copy.

Reader Service No. 103.

• Benzamidine-Sepharose 6B from Pharmacia is a new affinity chromatography gel for the rapid isolation of trypsin and trypsin-like serine proteases, for example, urokinase, enterokinase and kallikrein. Benzamidine-Sepharose 6B has a high binding capacity (binding approximately 13mg of trypsin per ml of gel), good flow properties, and is chemically and physically stable over a wide pH range. The gel can be regenerated and autoclaved repeatedly. Benzamidine-Sepharose 6B is designed for both laboratory and large-scale applications. A free data sheet on Benzamidine-Sepharose 6B is available on request from Pharmacia.

Reader Service No. 104.



Benzamidine-Sepharose 6B — stable over a wide pH range.

• Since the latest New England Biolabs catalogue was issued, a further 14 restriction endonucleases have been introduced, making the total 90.

Bsm I	5'...GAATC...3'	Nla IV	5'...GGNCC...3'
Dpn I	5'...GATC...3'	Nsi I	5'...ATGAT...3'
Dra I	5'...TTTAA...3'	SnaB I	5'...TACGA...3'
Fsp I	5'...TGGCA...3'	Spe I	5'...ACTAGT...3'
HinPI	5'...GCGC...3'	Ssp I	5'...AATAT...3'
Nae I	5'...CTAGC...3'	Sty I	5'...GAAAG...3'
Nla III	5'...CATG...3'	Xho II	5'...PACATCP...3'

Together with a growing list of methylases, which can be used in conjunction with certain restriction endonucleases to generate additional cleavage specificities, the number of cleavage specificities that New England Biolabs can offer is greater than 130. In addition to offering new cleavage specificities, a second goal has been to clone the restriction endonucleases so that the costs can be reduced.

Reader Service No. 105.

• Marfey's reagent, FDAA (1-fluoro-2, 4-dinitrophenyl-5-L-alanine amide) is the latest addition to the Pierce range of HPLC detection reagents. Optical isomers of amino acids can be simply and conveniently derivatized with Marfey's reagent.

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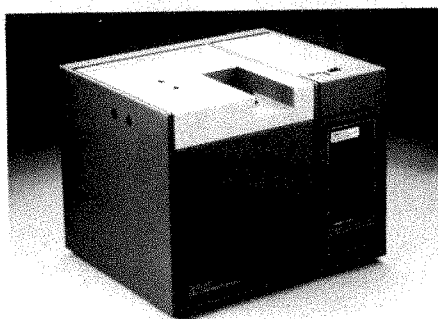
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gent; preparation is complete in 90 minutes. The amino acid derivatives can be easily separated and quantitated by reverse phase HPLC. Derivatives have an absorption coefficient of $\sim 3 \times 10^4$ and can be detected by ultraviolet light at 340 nm with picomole sensitivity.

Reader Service No. 106.

• Packard Instrument's new Model 436S satellite gas chromatograph features the same capillary performance as the Packard Model 436, but has no programming facilities and thus offers considerable cost savings over conventional GCs. It is designed to function in a Packard GC network system for routine process and quali-



Packard's Model 436S gas chromatograph. ty control applications. The oven has temperature gradients as low as $\pm 0.1^\circ\text{C}$, and excellent short- and long-term stabil-

ity. Other features shared with standard Model 436 include high-speed electrometers, proportional oven temperature control, microprocessor control and non-turbulent column environment. The Model 436S is ready to accept microbore columns without modifications.

Reader Service No. 107.

• Elsevier-Biosoft, a producer of biomedical microcomputer software, has recently increased its range of products to include an enzyme kinetics teaching program (ENZLAB), a highly versatile statistics package (STATSTREAM) and an expert system shell (EXPERT 4), among others. Readers are invited to write for a copy of the *Biosoft News* newsletter for product information. Elsevier-Biosoft is also pleased to hear what other programs readers would find useful to have available. Approaches are also welcomed from prospective authors and a copy of *Guidelines to Authors* is available on request. Write for details, mentioning *Nature*, to Elsevier-Biosoft, 68 Hills Road, Cambridge, CB2 1LA, UK.

• The Epsilon IgE enzyme immunoassay from Beckman Instruments, Inc. with one-step antibody incubation reduces hands-on time for fast turn-around. The test technology provides outstanding specificity, sensitivity and precision of IgE measurements in human serum. The simplified Epsilon IgE assay uses established EIA methodology with a time-saving easy-to-learn protocol. Tubes are set up once for a single antibody incubation step and washed once. No sample preparation or dilution is needed and 30 + 30 minute incubations allow fast reporting of results. A multi-site sandwich methodology is used. A monoclonal antibody on the bead picks up IgE providing specificity. A polyclonal antibody conjugate attaches to multiple sites on the IgE molecule for greater speed and accuracy. The Epsilon assay has a dynamic range of 10 to 400 IU ml^{-1} , excellent correlation with RIA proc-

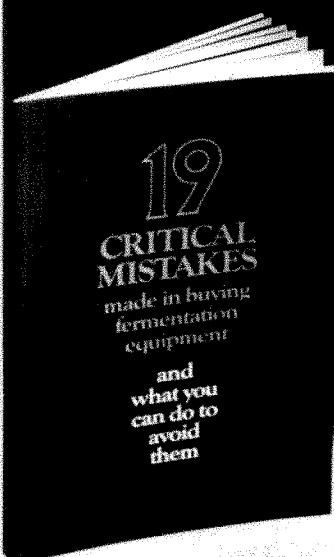
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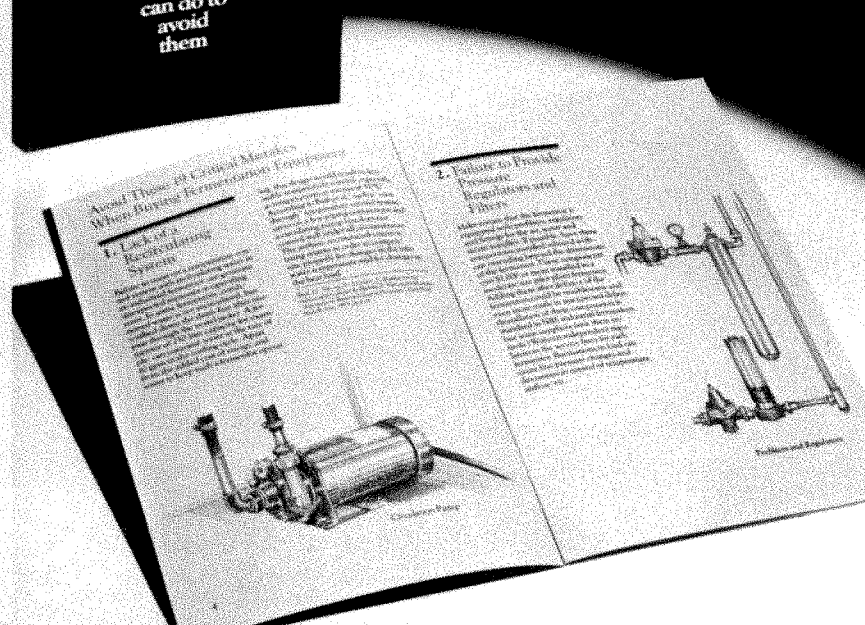
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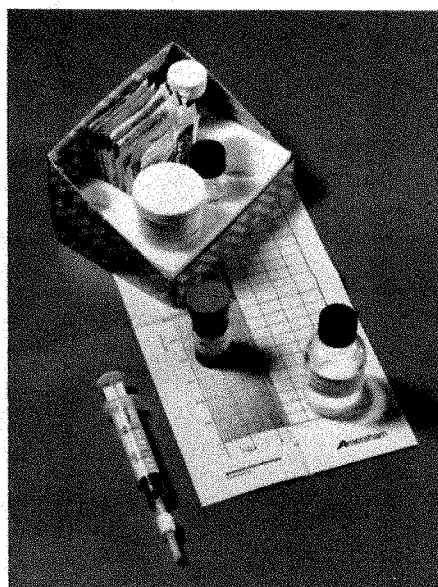
Reader Service No. 108.

• A new wall chart available from Boehringer Mannheim, *Coenzymes Data for the Laboratory*, is a reference guide which includes, among other information, the absorption coefficients and quotients, the pH in aqueous solution, and the molecular weights of the pyrimidine cofactors, NAD(P)H. Another chart, recently updated, features *Proteases and Protease Inhibitors*. This poster contains information on over fifty proteases, inhibitors, and chromogenic substrates including specificity and pH optima. Anyone involved with protein chemistry, protein sequencing or the prevention of the proteolytic activity will find this chart a handy and useful reference.

Reader Service No. 109.

• Newly introduced manometric modules for HPLC and LC give a pulse-free flow and a digital pressure readout. The pressure reading is transmitted directly to a recorder or to a computer. These modules from Gilson Medical Electronics, offer continuously variable high and low pressure limit adjustments. They stack onto and draw their power from the Gilson 302 or 303 pumps. Three different models are available: the 802C is designed to complement Gilson pump operations at high pressure and analytical flow rates, the 803C is for use in preparative HPLC systems, and the 804C is ideal for preparative LC. Also new from Gilson is a range of fixed-volume pipettes, Microman MF. Especially designed for the clinical chemist, Microman MF simplifies routine laboratory work. Based on the principle of positive displacement, Microman MF is well adapted to high or low density liquids or viscous samples. Typical applications include serum, whole blood and solvents. Eight different models cover the range of 5 to 200 μ l.

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A new research kit from Amersham to assess the status of vitamin D.

• Vitamin D status can be assessed simply and accurately using a new research kit from Amersham. The kit measures 25-hydroxy-vitamin D, which makes up 80-90% of total vitamin D metabolites in serum. The assay system uses a competitive protein binding assay and a tritium tracer. Sep-pak cartridges are provided for simple and effective sample purification. The assay range is 2-32ng ml⁻¹ and each pack contains sufficient material for the construction of one standard curve and the assay of 20 unknowns in duplicate. Combined measurements of both vitamin D₂ and D₃ series metabolites are made. The use of Sep-Pak gets round the problem of inaccuracy and unreliability inherent in methods based on solvent extraction alone, while avoiding HPLC or lengthy chromatographic steps. The cartridges can be used with ordinary laboratory syringes if required. Amersham already produces an assay for 1 α -25-dihydroxy vitamin D.

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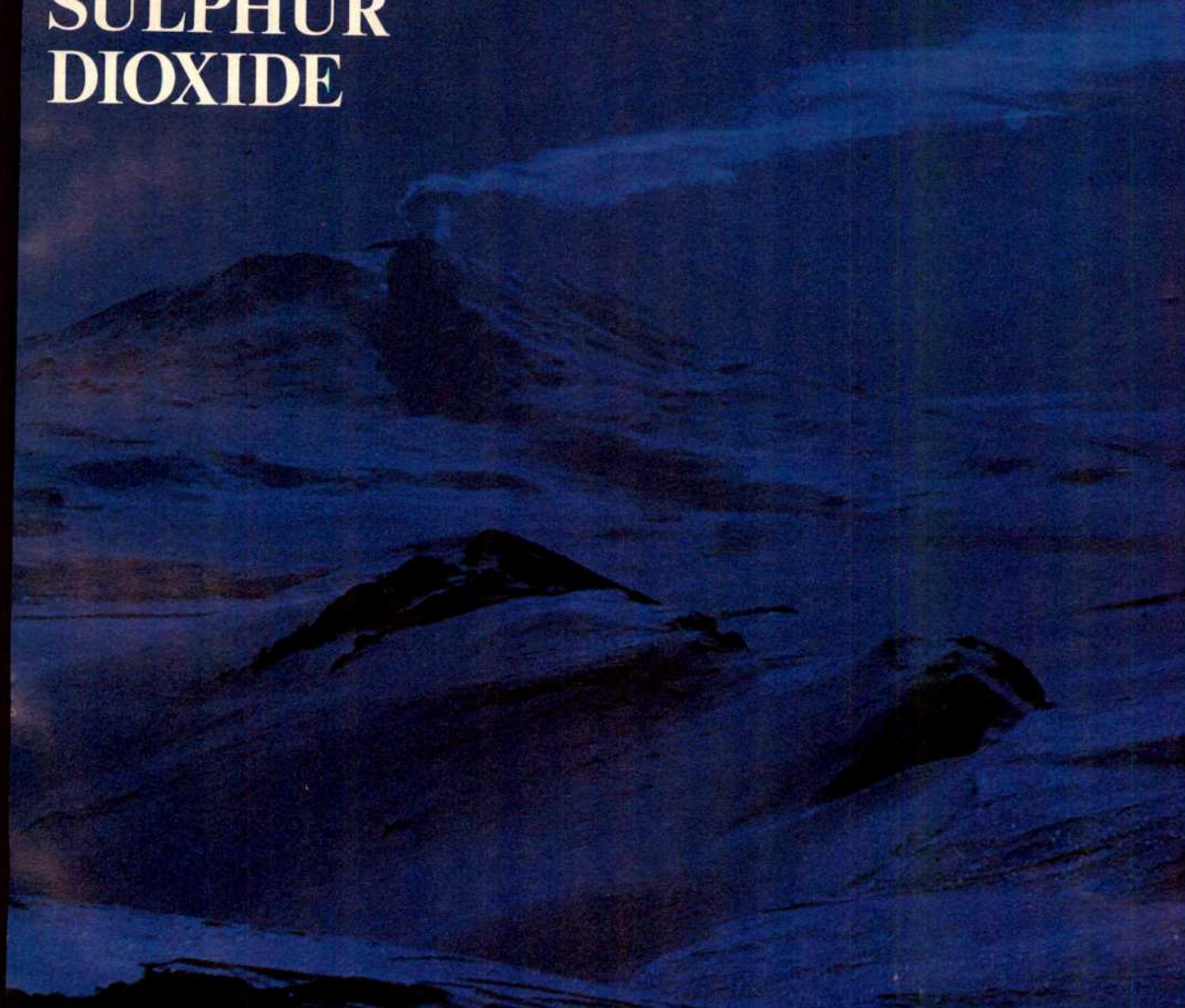
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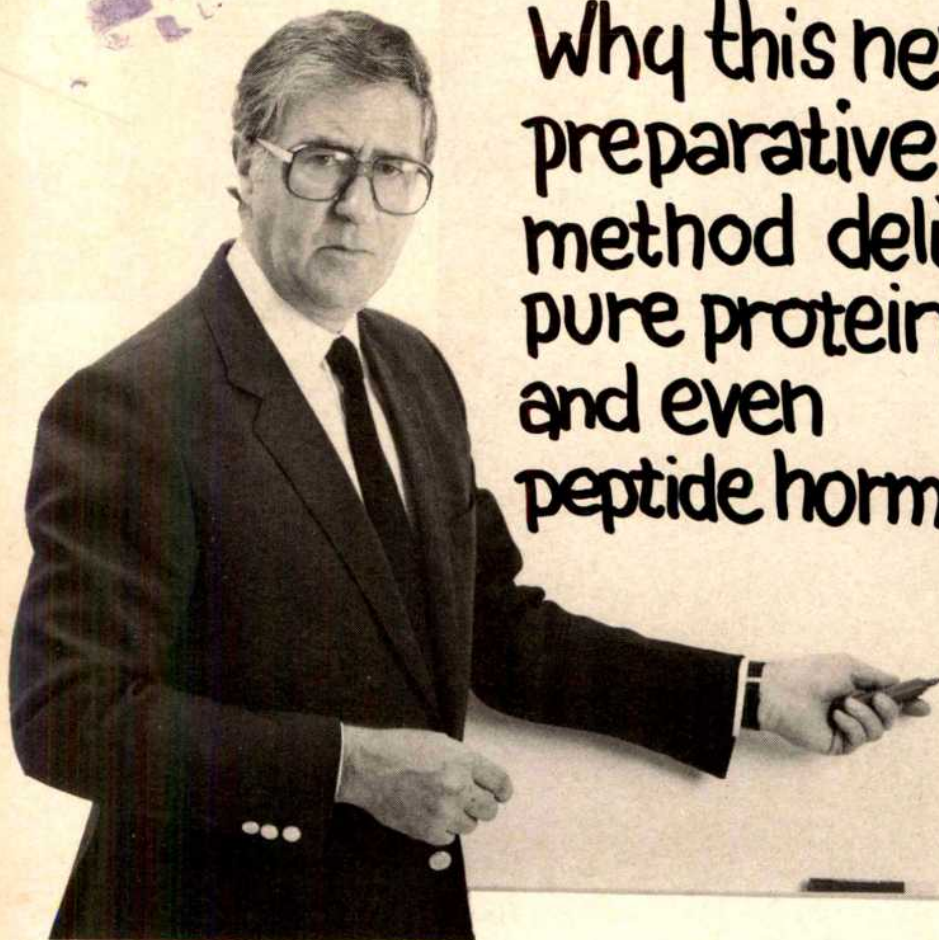
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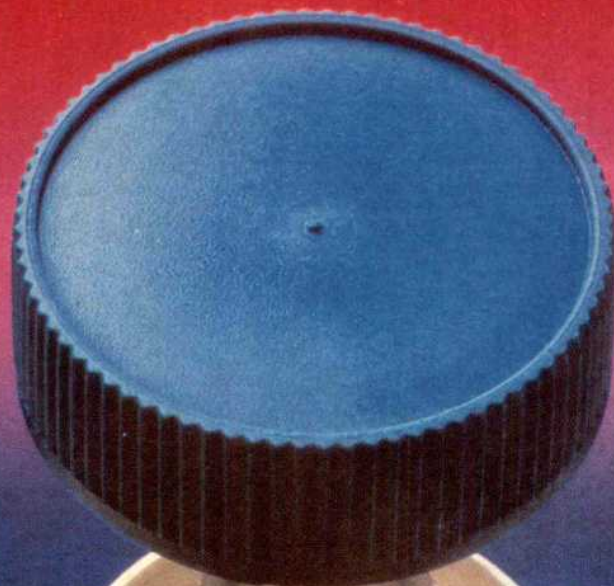
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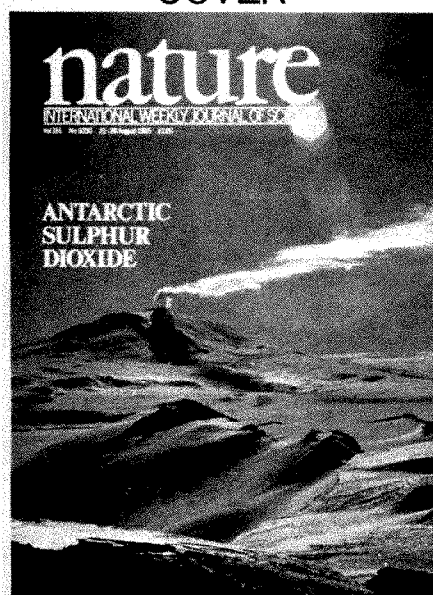
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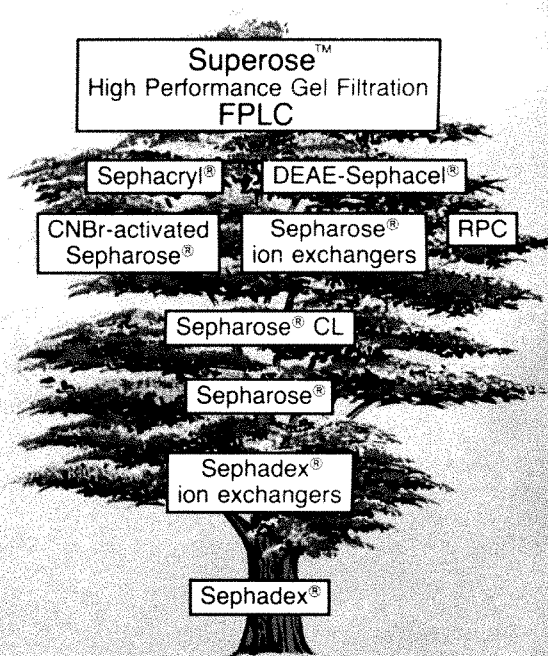
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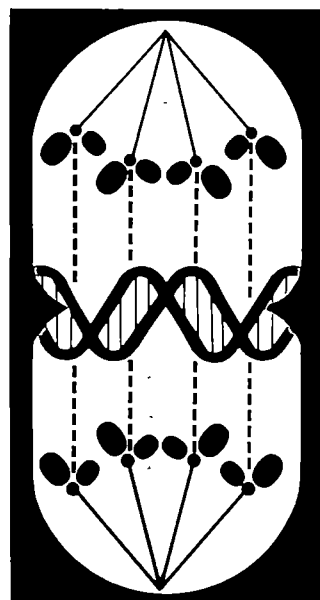
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(Tübingen)
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Topics
Vertebrate neural systems

Early development—
Xenopus

Early development—
Drosophila
Development of invertebrate neural systems
Caenorhabditis

H.R. Horvitz
(MIT)

DAY 2: TUESDAY, OCTOBER 8

CELLS

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(Stanford)

Topics
T-cell receptors

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(U. of Washington at Seattle)
J.M. Bishop
(U. of California at San Francisco)
M. Ptashne
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I. Herskowitz
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B cells

Oncogenes

Regulatory proteins
Yeast development

E. Jaworski
Monsanto

Plant transformation

DAY 3: WEDNESDAY, OCTOBER 9

GENES

Speakers
R. Palmiter
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(Stanford)
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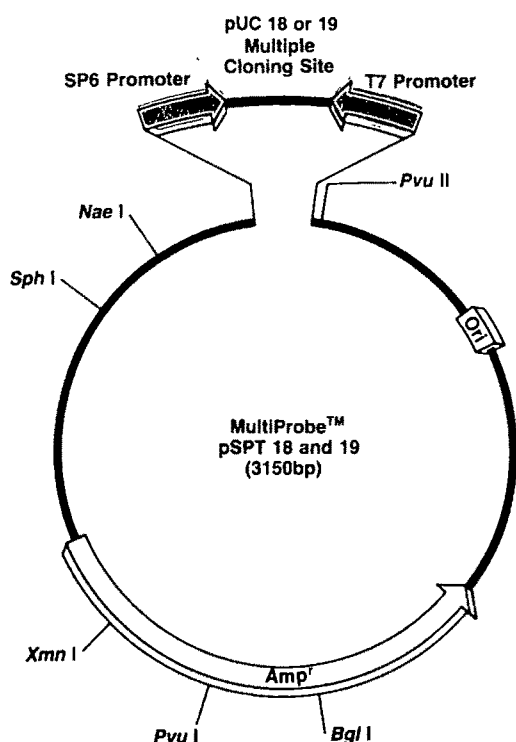
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No more NPT rows, please

The third review conference of the Non-Proliferation Treaty opens next week at Geneva. Old discontents are bound to surface. Can the participants think forward too?

WITH the passage of time, the Non-Proliferation Treaty (NPT) becomes increasingly important. Of all the extant instruments of arms control, it is the only one to include a substantial number (nearly 130) of signatories whose signatures imply self-restraint (as distinct from formal acknowledgement that others are restrained), it is the chief means by which it is possible to distinguish would-be bomb-makers from the rest and it has the virtue of providing a public discussion of nuclear arms control at regular intervals of five years. The third conference in this series, which begins next Tuesday (27 August), is certain to be as wide-ranging as its predecessors. The hope is that it will not be as rowdy as that in 1980, when the conference failed to draft an agreed statement.

The participants seem to have learned a great deal from that fiasco. On this occasion, people seem to have been diligent in their preparation for the review conference. In the hope of providing some kind of structure for the inevitable debate between the non-nuclear powers on the adequacy of the steps taken or attempted towards nuclear disarmament, there is to be a separate committee on the topic. Similarly, the safeguards system and the peaceful use of nuclear power are to be dealt with in an organized way. None of this will prevent complaints or recriminations; but since there is no reason to think that any of the participants wants the NPT to collapse, there are grounds for hoping that vituperation stops short of vilification.

The NPT is often described as a bargain between the nuclear and non-nuclear powers, but it is more than that. The three overt nuclear powers (France and China do not belong) have agreed to search for agreements on the reduction of their nuclear weapons, while the signatory non-nuclear powers have accepted international safeguards as an earnest of their promise not to make them. The treaty also lays down that the nuclear powers will provide technical assistance with the development of civil nuclear power in non-nuclear countries, or at least offer access to the International Atomic Energy Agency (IAEA). Parties of both kinds promise self-restraint, which is where the bargain rests. But both kinds of parties also have what must be supposed to be an identical interest that the number of nuclear powers should not increase. Only that common interest will enable them to hang together in a year when the two principal nuclear powers have nothing substantial to report except that they have reconvened, perhaps in a more hopeful environment, a series of negotiations broken off halfway through the interval since the 1980 conference.

Even so, the non-nuclear powers had better bite their tongues when complaining at Geneva that the nuclear powers should have done more. There are some minuses but also, surprisingly, some pluses. The minuses are that the full extent of the Soviet deployment of SS20 missiles has become apparent, that the countervailing modernization of NATO forces in Western

Europe has begun and that the United States has created at least confusion, perhaps justifiable alarm. Certainly there will be voices raised at Geneva during the next four weeks about the folly of this proposal, of which the best that can be said is that it cannot be as effective as it claims; and many of them will be Soviet voices. The pluses are more easily overlooked. Many governments will have forgotten that in 1980, the Carter administration in the United States had signed the second part of the SALT agreement on strategic weapons, but had not taken it to Congress for ratification, perhaps because it dared not risk defeat, perhaps because of the reason given that Soviet dealings with Afghanistan were an offence. Relations between the two superpowers may not be all they should be, but they have improved a great deal since 1980.

On the issue of civil nuclear power in non-nuclear weapons states, the climate has also improved or has become more realistic. The non-nuclear powers have put behind them the disappointments (for some) of the UN conference on science and technology for development, have put such resources as they have been able to spare for energy production into energy conservation (which always had first claim), but have begun to find opportunities for the economic use of nuclear power; the signs are that the international supply system, stripped of the over-optimism of earlier decades, is working well. Even the safeguards system, much resented by non-signatories such as India, is working more smoothly and less obtrusively — but there is still a need that the details gathered by IAEA inspectors should be made public.

So why are people still worried about the proliferation of nuclear weapons? NPT is an important part of the system for preventing proliferation, but is only a part of it. And during the past five years, there has been literally no progress in persuading those outside NPT to join with the other signatories. Israel remains putatively a nuclear power. Pakistan and India plainly seek the same model. Argentina and Brazil have not ratified the treaty. Nobody can guess what Libya let alone South Africa is about. China and France are conspicuous by standing aside. That is why one of the chief objectives for the next five years should be to persuade backsliders like these to join NPT. The task is political, but the benefits would be great. □

What hope for AIDS?

The temptation to legislate to protect society from the spread of AIDS should be resisted.

HARD cases make bad law, and public anxiety is a poor basis for social legislation. That should be the starting point for any consideration of how government should respond to the demands popping up all over the world that something should be done to protect people from AIDS, otherwise acquired immune deficiency syndrome, now known to be caused by the virus HTLV-III, or LAV in France. That there should be a certain air of panic is understandable. AIDS is a dreadful and also, still, a mysterious disease. Only some 10 per cent of those infected with the virus develop the symptoms that lead almost always to death from adventitious infection, for reasons not yet understood. Little is known, as yet, about the efficacy with which those

Biological manuscripts

Contributors are reminded that, with the transfer of the Biological Sciences Editor to the Washington office, they should in future send **four copies of all manuscripts** offered for publication either (as at present) to London or to Washington.

carrying antibodies against the virus can infect others. Moreover, the death tolls mount. More than 5,000 people have already died in the United States. In Britain (where mortality exceeds 100), other European countries and Australia, while the numbers of those dead are still small, there is the awkward prospect of a rapid increase as the larger numbers of patients with overt disease eventually succumb. It is bound to carry weight with people everywhere that in New York, among males in their thirties, AIDS is reported to have taken precedence over homicide as the chief cause of death. Where will it end, people are asking — and in the meantime they demand protection for themselves from the disease.

That is why it is important that everybody should understand how little can, at this stage, be offered. AIDS is a tragic disease, but a collective tragedy. Its emergence is a piece of evolutionary bad luck for all of us. Although still largely confined to male homosexuals, AIDS is certain in due course to spread more widely (see *Nature* 312, 97; 1984). This appears to have been the case in Zaire since 1977, for retrospectively it has been shown that the virus has been widespread and heterosexually transmitted for the best part of a decade (Piot *et al. Lancet* ii, 65; 1984). Already in the United States, there are reports of some scores of infant deaths, presumably as a consequence of congenital infection. There is nothing to suggest that AIDS will not become just another venereal disease, but one not tractable to the antibiotics familiarly now in use. Fortunately there is as yet no evidence that the virus is transmitted by means other than sexual intercourse of one kind or another.

What, in these circumstances, should public policy be? Plainly there is an urgent need that strictly adventitious infections should be avoided. The cases of haemophiliacs infected through contaminated blood are doubly tragic; people disabled by an inherited genetic deficiency are cruelly dealt with if society cannot provide them with a safe palliative. There is the strongest reason why haemophiliacs and others in need of blood transfusions should be protected as far as possible by the use of the screening assays so far developed; there is even a case that those discovered to have HTLV-III antibodies in their blood should be identified, warned of their condition and black-listed as blood donors. So much is feasible and urgently necessary. Governments should not stint themselves. In Britain, the government should not expect hard-pressed health authorities to provide the extra funds they will need out of budgets already trimmed to the bone.

How much further is it possible, or wise, to go? There is a natural temptation to make the disease notifiable in the technical sense, which would give health authorities the power almost automatically to confine patients in isolation; quite apart from the lack of isolation facilities that would be required to keep patients out of contact with other people for months on end, the result would almost certainly be that those most at risk would be deterred from seeking medical help for suspected infection. Sensibly, most governments and the British in particular have resisted this course of action. Others, however, would go even further. Dr John Griffin, director of the Association of British Pharmaceutical Industry is reported by *The Times* to have written urging that there should be compulsory screening of male and female prostitutes, groups almost certain to be or to become foci for the spread of infection. The obvious difficulty is that there can be no effective way of enforcing such legislation. How is prostitution to be defined? What assurance can there be that, given a workable definition, the result will not be that prostitutes are even more firmly put out of touch with physicians? These are problems that those seeking to control the venereal diseases that already exist have grappled with in vain for years. Even though AIDS is a sombre shadow over public health, there can be no reason why these diseases should be more easily conquered now than in the past. In any case, not nearly enough is known about the transmission of AIDS for it to be more than a presumption that prostitution is such a crucial link in the process. Legislation would probably do more harm than good. Public education is the better course.

The message that needs to be made public is unusually depressing. Prophylactic vaccines are a long way off. The prospects for curing people who have developed the disease are even more distant. Palliation is uncertain, as things are. How is it possible to inform the general public about an infectious disease for which so little can be done? There are two cheerful things to say. First, it is a mere four years since AIDS was recognized as such, since when the infecting organism has been identified by heroic efforts in the United States and France; at least diagnostic kits for AIDS antibodies now exist, and will be improved. And, by good fortune, the lymphocytes which are the targets of the virus are also at the centre of one of the most rapidly moving fields of research, from which it is not unreasonable to hope that ways of manipulating the process of infection will spring. But, second, there has also grown up, in this short time, experience of how patients can be helped psychologically to come to terms with their condition, and with the importance of behaving so as to make an individual contribution to the process of trying to slow down the spread of the disease. The public clinics in the United States have mostly shown a remarkable blend of compassion and hard-headedness in their care for patients. The pity is that so many of the communities in which they work are over-zealously concerned to seek personal safety in illiberal legislation. □

Academic independence

The British government's inquiry into the University Grants Committee could be useful.

THE British government's plan (see p. 669) to review the constitutional status of its University Grants Committee (UGC) should not provoke the knee-jerk reaction that comes first to mind. Since 1919 the University Grants Committee has been a buffer between the government and the universities it supports. For most of the time, the committee has been applauded as a unique (and even uniquely British) device for spending public money without running the seemingly inevitable risk of government interference. (The British Broadcasting Corporation enjoyed a similar reputation until its governors caved in three weeks ago to an ill-judged request from the Home Secretary not to broadcast a film on Northern Ireland, and until it leaked out that BBC appointments are regularly vetted by the government's intelligence services.) Certainly, the existence of UGC has ensured that the affairs of individual universities are not open to scrutiny by, for example, the House of Commons, which has suited both the university system and successive governments. Broadly speaking, the committee's recommendations about the development of the individual universities as well as the system as a whole have also been fair and constructive. The fly in the ointment is that UGC's seeming independence has always been more apparent than real. During the six years of the present government, ministers have chosen to remind the universities of the old doctrine about the right of the one who pays the piper to call the tune. The committee's independence has been undermined. There is at least a chance that the inquiry now to be mounted will restore some of that independence.

Constitutionally, UGC is not an executive body but yet another advisory committee. Each year it makes recommendations to the Department of Education and Science about the distribution of the funds available, whereupon the government writes the appropriate cheques. There has never been an occasion when the government has rejected UGC's advice, but constitutionally there is no reason why that should not happen. Over the past decade (since before the present government took office), UGC has lost the essential benefits of the old quinquennial system under which the scale of spending was fixed in outline five years in advance; now there are three-year rolling forecasts, which may be changed from year to year. That was the rubric under which the government arbitrarily announced, at the end of 1980, that the total budget of the universities would have to shrink by 8.5 per cent over three years; the UGC of the time incurred the wrath of those universities whose budgets

were disproportionately made to shrink, which was a convenience for the government which had already decreed what fees should be paid by students from overseas. After all this buffeting, UGC lies in a constitutional limbo made more ambiguous because it has no staff of its own, and no freedom to spend money on the techniques of running an office.

The biggest challenge to UGC's independence lies only a little way ahead. The committee has made no secret of the opinion that the time will soon arrive when it will be prudent for at least one of the universities in the British system to be closed. The overall budget is still shrinking by about 1.5 per cent a year in real terms, while savings that may arise from a more selective distribution of funds will not come soon enough to ease the pressure. Traditionally, in such a circumstance, it would be for the committee to say which institution should be shut, but it has plainly said that it will leave that invidious decision to Sir Keith Joseph, Secretary of State for Education and Science. The reasons are plain. Given that the British government is the ultimate paymaster, there is no chance that a decision to close a particular university could fail to become a political issue. There will be rows in the House of Commons, while the government itself will be tempted to make electoral calculations when deciding which parliamentary constituencies to offend. When that time comes, probably before the new inquiry is complete, it will be generally apparent that UGC is not in charge of the British university system nor even a universal buffer between it and the government. At that stage, the government will also be in a mood to regret that it has so emasculated UGC's independence.

This is an opportunity that British universities should exploit. The government's interest in the past few years has extended beyond budget-trimming to schemes for encouraging more concentration on science and technology and attempts to create a closer interaction between universities and industry, partly for its own sake but also in the hope of saving money. UGC has responded within the limits of its means as positively as could be expected, but the government has found that the provision of more places in science and technology cannot be engineered out of the blue, at no extra cost. Sir Keith Joseph has insisted on his government's right to shape the university system in ways like this, and within the bounds of what is possible, the claim cannot be denied. But the government cannot ask for what is not possible, nor can it, without the advice of people who know the university system intimately, know what it possible and what is not. From these considerations, the Butterworth committee's recipe for change should follow immediately.

First, UGC should be constituted as an independent public corporation, with the resources to manage its own affairs and the freedom to employ its own staff (mostly, at present, on loan from the department, but with increasing help from the universities). Second, it should be made financially autonomous, with the power to write its own cheques in favour of the institutions in its care — and the equally important right to carry over money from one year to the next. (Ideally, UGC should be able to accumulate the kinds of trust funds on behalf of British universities in general which do not at present exist.) But how to take account of government policy on universities? Simply by requiring that the membership of UGC should include the ministers responsible, the Secretary of State for Education and Science or his deputy, perhaps other heavyweights as well. Conventionally, of course, ministers do not belong to committees of which they are not the chairmen, but that would in this case be inappropriate. The record of the past few years shows that ministers have much to learn about higher education as a whole; membership of UGC should be quickly educative. But UGC itself is a sleepy and over-cosy committee, inclined to regard the British government's decisions about budgets and the like as slings and arrows of outrageous fortune. Its meetings would be more lively and more pointed if the perpetrators of these insults, real or imagined, were to be found around the same table once a month. And by what other means can there be a genuine partnership between the British government and the academic community on the management of the university system? □

Justice by bankruptcy

The Manville solution for the settlement of claims by asbestos victims could become a precedent.

THE Manville Corporation of the United States has had an eventful, no doubt salutary and (for the rest of us) admonitory year. Historically, the corporation has been a processor of asbestos and a manufacturer of asbestos products. In the past decade it has found not merely that its business has declined as the use of asbestos in the construction industry and elsewhere has declined, but that its existence as a commercial corporation had been put in jeopardy by the volume of the claims by private individuals and their families alleging that their health had been damaged by asbestos fibres, which are known to be capable of causing a characteristic form of lung cancer. Three years ago, no fewer than 60,000 lawsuits had been laid against the company, which took the unusual but proper step of seeking protection under the US bankruptcy laws. The company now guesses that the total amount of the outstanding claims amounts to more than \$2,500 million, more than it could hope to raise by liquidating its assets. So, if the suits were to have been taken seriously, the company was technically in the position of not being able to meet its creditors' claims out of its own resources, which is the same as to say that it was technically bankrupt. But to have sold up would have been of little help to the claimants, which is why the company sought protection under Chapter XI of the bankruptcy act, which allows a company to continue to run its business under court supervision, in the hope of generating the funds with which to pay off its debts. (Companies and also creditors elsewhere may envy this enlightened legislation.)

The outcome of Manville's flight to the shelter of the bankruptcy laws is nevertheless surprising and, in some ways, ironic. Part of the difficulty in dealing with the lawsuits has from the outset been that nobody could predict how many lawsuits were yet to be laid by people in whom overt signs of damage were not yet apparent. But how can the accountants take account of imponderables like this when trying to decide whether a company is technically bankrupt? The courts have fortunately devised a way, one that is almost certain to be followed in other such circumstances. The principle is that legitimate claimants against the company shall be compensated not by the outcome of the particular lawsuit they may have laid, but by means of payments from a trust fund Manville will be required to set up. First, there is a substantial sum due from Manville's insurers (big enough to have caused trouble on the Lloyds insurance market in London). Manville itself is required to bless the fund with \$200 million in cash and if necessary to hand over 20 per cent of its annual profits. The trust fund is also to own a substantial part of the company and there is a complicated arrangement about convertible preference shares that will, according to the volume of legitimate claims eventually made, allow the fund to finish up owning as much as 80 per cent of the company. The issue will be decided next month.

That, of course, is the irony. Manville and its existing shareholders are being required to do the decent thing in financial terms by its past and future victims, but in a way that gives them an interest in the success of the business. The potential advantages are, however, important. Settlements should be quicker. Less of the funds available for compensation will stick to the lawyers' hands by way of contingency fees. And it will be easier for people who think they may have been damaged to tell where they stand. If the small flurry of lawsuits being brought against tobacco companies on the grounds that cigarettes cause lung cancer should stick, other companies than Manville will be rushing off to the bankruptcy courts. The obvious difficulty is that the feasibility of a tobacco company continuing to trade would depend crucially on an actuarial calculation of whether the cost of compensating victims would suck up all the profits from selling cigarettes to those who escape. The moral, for those who must smoke, is to switch to a brand made by a manufacturer whose business is not solely tobacco-based. □

Radiation exposure

British nuclear workforce assessed for risks

RESULTS of a study carried out by the British Medical Research Council (MRC) claim that there is "no significant risk to health" from radiation to people working for the UK Atomic Energy Authority (AEA). The study, published last week in the *British Medical Journal* (291, 435 and 440, 1985), relates mortality to recorded exposure to ionizing radiation for 39,546 employees of AEA between 1946 and 1979. Of the 3,373 deaths that occurred, 937 were from cancers; overall, death rates of the sample population were lower than those in the country as a whole, but consistent with those expected in a normal workforce and probably accounted for by the social class of AEA employees. But one type of cancer, prostate cancer, was unusually prevalent in the sample group.

The UK study was undertaken as a result of a report in 1977 from the United States, which suggested that the risk of developing cancer after exposure to low-dose ionizing radiation is 20 times greater than the recommendations of the International Commission on Radiological Protection (ICRP). That study, which investigated a workforce in the US nuclear industry, has been criticized for its methodology and analysis of results. AEA therefore asked MRC to commission an independent survey of mortality in its own workforce. MRC's epidemiological study was performed by Professor Geoffrey Rose, Dr Valerie Beral and colleagues at the London School of Hygiene and Tropical Medicine. Mortality of all AEA employees since 1946 was examined and the results compared with ICRP's figures.

The authors of the new study say that their data give no unambiguous guide to the feasibility of estimating the radiation risks to people exposed to low doses by extrapolation from the mortality among the survivors of atomic bomb explosions, exposed to acute doses, the basis of the ICRP recommendations. The correlation between radiation exposure and mortality is so uncertain that, at the 95 per cent confidence limits, the data are consistent with a chronic radiation risk 15 times greater than that suggested by ICRP and, at the other extreme, a risk which decreases with increasing dose.

Of the deaths that occurred, leukaemia rates were slightly above the national average, but as the total number of deaths from this cause was small (35 overall, or 4 per cent of all cancer deaths) the excess could be explained by chance fluctuations. Mortality was not, in general, correlated with radiation exposure, the exception being prostate cancer, which caused 38 deaths. This figure is several times greater

than the national average in the small groups of workers who had relatively high exposure to radiation and who had also been monitored for contamination by radionuclides, particularly tritium. No explanation for this result is suggested by the *British Medical Journal* papers, which point out that tritium is not prostate-seeking, but that tritium contamination may be an indicator of some other agent.

MRC is now "actively considering" how to investigate further the results on prostate cancer. The epidemiological study is being extended for a further five years,

partly to obtain more reliable statistics and partly because, as people in the sample become older, more cancers will emerge which will allow risk estimates to be recalculated with greater precision.

Over the next few years, other UK studies on the effects of radiation will attain the stage that this MRC/AEA survey has reached. Studies in progress are on atomic weapons research staff, staff of British Nuclear Fuels Ltd (preliminary results already published), the National Radiological Protection Board's radiation register for the 1970s and the Central Electricity Generating Board's survey on its workforce. Pooling of these results with the existing and future AEA figures from surveys in other countries will enable more confident limits to be set on radiation safety levels and provide a more accurate estimate of the specific risks involved.

Maxine Clarke

Japanese economy

The future is even brighter

Tokyo

JAPAN's economic prospects will be even brighter in the years ahead. That is the message of the latest white paper from the Economic Planning Agency. And although the document repeats the familiar self-criticism of recent years that Japan lags behind the United States and Europe in fundamental science and the development of original technology, the criticism is only muted.

Indeed, the report predicts an economic future for which most European leaders would sell their souls. The past ten years — in which annual growth has regularly been above 5 per cent, two or three times that recorded in the United Kingdom, and in which Japan has emerged as the world's second greatest economic power — is dismissed as an era of "stagflation". From now on, Japan will enter a new age of inflation-free rapid growth.

Three factors will contribute to this expansion. The first is rapid growth in the telecommunications, electronics and other high-technology industries. In 1984 alone, Japanese corporations invested five thousand million yen (10^{12}) in these areas. Second is the growing service sector and increasing urbanization, with the Japanese population becoming ever more concentrated. And third is the growth of Japan's Pacific neighbours — from California through Australia round to Taiwan, Korea and China — which, it is believed, will make the Pacific basin the centre of world economic activity by the turn of the century, leaving the Atlantic nations to wither in their grey and sunless climes.

Only one small cloud floats in this perfect blue sky. Japan now supports a smaller percentage of old people than European countries. But by the end of the century, with the Japanese now the world's longest lived people, the situation

will have reversed and the nation will be faced by huge pension and medical bills. The report suggests that this may be dealt with by raising from 60 to 65 the age at which citizens can receive government pensions and by restricting the number of doctors. And that in turn has led to stern criticism that economic planning agencies have no business deciding welfare policy.

Alun Anderson

More money for NIH

Washington

IN the last days before its summer break, the US Congress defied the administration by passing a bill instructing the National Institutes of Health (NIH) to award no less than 6,200 extramural research grants in the current fiscal year, slightly less than Congress originally intended but 1,200 more than the administration's planned figure of 5,000 grants.

Thwarted by Congress's limitless generosity to NIH, the administration had tried to commit some of the 1985 budget for the future years of multi-year grants, a move that was challenged on legal grounds by the US Comptroller General. The supplemental appropriation now passed by Congress (HR2577) does not give NIH any money above the \$938 million they had already been given for research grants, but by specifying the number of grants, it prevented the administration from pursuing the multi-year funding plan (see *Nature* 313, 377; 1985). The legality of the administration's plan has not been resolved.

Researchers should not expect their money too soon, however. By denying his signature, President Reagan could yet exercise a "pocket veto" and kill the bill, as Congress is not in session to oppose him.

Tim Beardsley

US engineering

Backing for long shots

Washington

RESEARCHERS who fail to win a grant from the National Science Foundation's engineering directorate will not in future be able to blame the foundation's excessive caution or lack of vision. The dynamic new assistant secretary for engineering, Nam Suh, has instituted a new grant for proposals that are rejected by the peer review system but which are so exciting that they perhaps ought to be funded just in case. This year, 12 such "high risk/high return" projects have been supported, with grants worth a total of \$1.2 million.

The new grant is administered by a special 7-member Committee on Innovative and Exceptional Research (to distinguish it, presumably, from the rest of the research supported by the foundation). The committee's chairman, William Butcher, explains that the grant was established to counter a fear that peer reviews of research proposals are inherently biased against "way-out ideas". Besides funding risky proposals, the committee can consider "highly innovative truly exceptional proposals" (it has not yet received any of these) and, for the sake of completeness, proposals that do not fall into any of the other categories (so far, they all do).

This year the committee was unable to use all the \$2 million at its disposal, perhaps because the scheme only got under way in March, but next year Suh (himself a former grant holder) is planning to put aside \$3-4 million for off-beat ideas, two per cent of his total budget. Because this is less than allowed tolerances on spending, the scheme has not been formally sanctioned by the directorate's advisory board, although according to Butcher most members know about it and approve. The high risk projects supported this year include a scanning optical microscope with a resolution of 50 micrometres, which could be used to examine living cell membranes, studies of polymerization in supercritical fluids and a proposal to generate "squeezed state light", which one reviewer described as being potentially as important as the discovery of the laser.

Butcher emphasizes that the new grant does not eliminate peer review: all proposals are still put through the normal assessment procedure, but some that get mixed reviews and so are rejected by programme managers are given a second chance by his committee. Only one in three of the proposals considered by the committee were funded this year, but Nam Suh hopes that as the word gets around that his directorate will support off-beat ideas, researchers will be encouraged to be more daring in the sorts of applications they make. **Tim Beardsley**

US gene cloning

Academics barred from P4 lab

Washington

THE National Institute of Allergy and Infectious Diseases (NIAID) is to ban the use by outsiders of its high containment laboratory at the Frederick Cancer Research Facility in Maryland. The laboratory, which was much used in oncogene research in the late 1970s, is one of only a handful of laboratories with P4 rating, the highest level of containment facility, and research being conducted there may have to be reclassified at a lower level of confinement if it is to continue elsewhere.

The Frederick laboratory, although formally run by and for the National Institutes of Health (NIH), has in practice often been used by outside researchers who needed access to secure containment. For the past year, the laboratory has been used successfully by Jack Murphy of Boston University Medical Center to clone fusion genes consisting of a synthetic oligonucleotide coding for human alpha-melanocyte stimulating hormone and portions of the structural gene for diphtheria toxin; the research could eventually lead to a "magic bullet" treatment for melanoma.

Last week Murphy went to the subcommittee on toxin cloning of the NIH Recombinant DNA Advisory Committee (RAC) to ask permission to continue the work at a lower level of containment, and also to seek approval for developing under P4 containment chemotherapeutic agents that would kill T and B cells carrying interleukin-2 receptors. This work, to be done in collaboration with Terry Strom of Beth Israel Hospital in Boston, might allow the creation of specific immune defects to prevent rejection in transplant pa-

tients, because resting and "memory" lymphocytes do not express interleukin-2 receptors.

Myron Levine of the University of Maryland, one of the most liberal members of the toxin subcommittee, said the proposed new work should be done only under P4 containment; other members agreed, noting that the effects of chronic exposure to such agents would be unpredictable and potentially harmful. But the subcommittee was then informed by Malcolm Martin, department chairman of the NIAID molecular biology laboratory, that Murphy could no longer use the Frederick facility because of the institute's decision to restrict access to NIH employees.

Faced with the option of preventing Murphy's work altogether, the subcommittee decided that the transplant rejection work could go ahead under P3 conditions, while the melanoma work could be reclassified as a hybrid between P2 and P3 until more toxicity data are gathered.

Martin explained later that the decision to exclude non-NIH workers had been taken because of competition from intramural research and an imminent refurbishment. There is another P4 laboratory in the NIH division of safety that is still available to outside researchers, although the space available is too small for many purposes. But Martin said that to require containment higher than P2 for Murphy's work "doesn't make any sense" because the host bacterium, the disabled K-12 strain of *Escherichia coli*, has no potential to colonize humans. **Tim Beardsley**

Indian guided missile nearly ready

New Delhi

THE Indian missile programme has reached a crucial stage and an advanced guided missile is expected to be ready as early as next year. Earlier this month, Prime Minister Rajiv Gandhi and Defence Minister P. V. Narasimha Rao saw the missile at the Defence Research and Development Laboratory (DRDL) at Hyderabad. They also watched the simulated flight of the indigenously developed missile and the static testing of its motors. Mr Gandhi later laid the foundation stone for a £75 million research facility at a 1,000-acre site 22 kilometres from Hyderabad where the missiles will be "assembled, tested and checked out". The new facility is scheduled to be fully operational in three years, but it is expected to show results next year.

No details are available about the missile's capability or range or if it can carry a nuclear payload. But the development has been welcomed in India in the light of Pakistan's nuclear ambitions. According

to defence scientists, it will have solid and liquid propellant motors and will be "highly sophisticated". The propellants, inertial guidance and control systems have all been developed indigenously.

The missile programme is directed by Mr Abdul Kalam, director of DRDL and chief designer of India's satellite launch vehicle, the workhorse of the Indian Space Research Organisation (ISRO). Four years ago, Kalam was brought from ISRO to DRDL to develop a surface-to-air missile for the defence system that depended exclusively on missiles imported from the Soviet Union.

DRDO, under the directorship of Dr V.S. Arunachalam, who is a metallurgist, is also working on electronic warfare, early warning systems, radars, submarines and a main battle tank (MBT) for the army. DRDO recently signed an agreement with Italy for collaboration on electronic counter-measure systems.

K.S. Jayaraman

UK research councils

The selling of NERC research

THE smallest source of Natural Environment Research Council (NERC) income casts a disproportionately large shadow. Private sector and overseas government contracts account for only £3–4 million out of a £94 million budget; yet, as NERC chairman Hugh Fish emphasizes, "bolting on some consideration of commercialization" is an important priority for NERC strategic research. These activities give Britain's brightest scientific lights overseas experience, which Mr Fish thinks important, and, more to the point, "it is in the national interest that research should be applied as quickly as possible to earn the country money".

What NERC is selling is expertise in applying research to specific technical problems; in contrast, the marketability of hardware that stands alone is of almost minimal interest. Such a priority points to the developing world as an obvious NERC market, one which many of its institutes

are now exploiting

While some of this work is directly contracted with foreign governments, Mr John Morris, senior executive officer of NERC's marketing group, says that a preferred arrangement is a collaboration with a consultant group. The consultants are then responsible to the government or world-aid source for long-term monitoring, logistics and penalty clauses, leaving NERC only as a subcontractor of technical know-how.

The Institute of Terrestrial Ecology (ITE) is a NERC organization that has been involved in contract work for over 10 years. According to Professor Fred Last, ITE deputy director, ITE's experience with contracting has now given it the freedom to choose contracts that fit into its fundamental science programme. He is concerned, however, that few of these contracts have room for a supplement to support basic research; in real money terms, contracts have not yet made up for the decreases in the basic science budget, leading to a "burning up of the resources" on which contracts are based.

The organizational embodiment of NERC's commitment to marketing its strategic and applied research is its Research Marketing Group, which is divided into two parts. The first, which Mr Morris calls "science push", involves finding the NERC projects with exploitable applied research and then working out possible logistics, such as, for example, offering a commercial client the resources of several NERC institutes rather than a single project. The "demand-pull" side, on the other hand, keeps up with the priorities of world-aid funding agencies. Mr Morris claims that far from endangering NERC objectivity by private funding, NERC actually has an edge by offering companies what they do not want to hear as well as what they do. The research group, says Mr Morris, has not been around long enough to judge its effect in financial terms; but the group has had a clear influence in developing "an enthusiasm for a balanced push into commercialism" among NERC institutes.

Examples of this balanced push include efforts by the Institute of Marine Environmental Research to form a joint company for the assessment and exploitation of marine resources concentrating on low-level toxicity testing, marine surveys and mathematical modelling.

Another is the GLORIA sidescan sonar system for mapping the ocean floor, developed by the Institute of Oceanographic Sciences. In cooperation with the US Geological Survey, IOS has been using GLORIA to map the 200-mile economic exclusive zone off the west coast of the United States, with plans for this year covering the Gulf of Mexico and Puerto

Rico. As with other NERC marketing efforts, GLORIA is portrayed as essentially the selling of expertise.

A year ago, NERC, the largest single contractor for DG XII, the European Commission's directorate responsible for science research and development, set up a Brussels branch of its Research Marketing Group to liaise with the Commission.

Elizabeth Collins

British Association

Scottish meeting

BILLED as a festival of science, the annual meeting of the British Association for the Advancement of Science (BA) will be held on 26–30 August at the University of Strathclyde in Glasgow. Roughly 1,500 members are expected to attend, with day visitors and school parties bringing that number up to nearly 3,000.

Exploitation of research is the meeting's primary theme. Over half the papers offered will touch on this topic, including sessions on opportunities in British engineering, engineering in agriculture and the art of forestry. Applied science is also a year-round concern for the BA, as shown by its science and industry committee and increasing corporate interest in the organization. Corporate membership fees have risen more than fourfold over the past year, from £6,221 in 1983–84 to £25,850 in 1984–85.

Another principal theme of the meeting, waste disposal, will be covered by the BA debate on disposal of radioactive waste as well as papers on energy recovery from refuse incineration, geochemical aspects of acid rain, waste disposal and energy, and pollution in the sea. Those interested in the third theme, population, can attend lectures on the biosocial aspects of Scottish migration, breeding in the sea, urban policy issues, and contraception and the control of fertility.

Three BAYS (BA Young Scientists) lectures will be offered, on all-optical digital circuits and the future optical computer, vision and movement and superficial chemistry. Other activities have also been planned to interest the roughly 500 expected BAYS visitors to the meeting.

Sir George Porter will be elected president of the BA on the last day of the meeting. The BA budget has increased by roughly £34,000 this year, from £289,351 in 1983–84 to £324,330 in 1984–85.

Elizabeth Collins

Correction: New Zealand science

SPENDING on research and development in New Zealand in 1984–85 amounts to \$NZ200 million by government, \$NZ68 million by universities and \$150 million by industry. These figures were given incorrectly in the supplement on Science in Australasia (*Nature* 18 July, p.187). □

US troops and AIDS

Washington

THE US Department of Defense (DOD) will from 1 September require civilian blood banks to disclose the names of active-duty personnel whose blood tests positively for antibodies to the acquired immune deficiency syndrome (AIDS) virus. The blood banks have agreed only reluctantly to comply with the DOD request.

Blood banks such as the American Red Cross plan to inform potential military donors, before their blood is taken, that results of tests may be passed on to military authorities; details of how the policy will be implemented have yet to be settled. But the spectre of military donors refusing *en masse* to give blood as a result has become a source of anxiety for blood banks, some of which obtain a substantial proportion of their blood donations from servicemen.

DOD argues that knowing who has been exposed to the AIDS virus could be important for service readiness; for example, vaccinations given routinely to servicemen could be dangerous to those with immune deficiencies. Homosexual rights advocates, who represent one of the high-risk groups for AIDS, protest that unless kept confidential, information from AIDS antibody tests could be used to stigmatize or discriminate against those who have evidence of antibodies, even though many of these may not be infectious and only a small proportion are likely to develop overt disease. The link between AIDS and intravenous drug abuse might also lead to stigma. But DOD, which is separately considering its own mass screening of military personnel for AIDS antibodies, says that test results will be used only for medical evaluation and counselling.

Tim Beardsley

Japan to Halley

Second comet probe launched

Tokyo

AFTER several days of delay because of bad weather, Japan's main Halley's comet probe (Planet A) finally had a perfect launch from the Uchinoura Space Centre, Kagoshima, on the morning of Monday 19 August.

The satellite was carried aloft by a 28-m tall Mu 3SII solid-fuel rocket at 8.33 a.m. Some eight minutes later, each of the rocket's three stages had separated according to plan and the final "kick" stage had injected the satellite directly into its orbit. Test transmissions were picked up shortly afterwards by National Aeronautics and Space Administration (NASA) tracking stations in California and in Japan, indicating that the launch had been faultless.

The 135 kg satellite, given the name *Suisei* (Comet), will continue into orbit around the Sun. By March next year it will have completed three-quarters of its first revolution and rendezvous with Halley's comet soon after perihelion. Also on hand will be the Japanese satellite *Sakigake* (MST5), launched in January this year and at present orbiting the Sun, waiting for March.

In March, *Suisei* will pass within a million kilometres of Halley's comet and take a series of vacuum ultraviolet (Lyman alpha line) images of the comet's coma as it passes perihelion. This stage of the comet's journey is of particular interest, for water and other volatile materials leave the comet nucleus and are photodissociated through a complex series of intermediate radicals. The UV images should make it possible to identify exactly what stages are involved.

Suisei is rather small compared with the satellites from other nations that will be observing Halley, and everything possible has been done to save weight. The satellite has not been fully stabilized by a three-dimensional attitude control system but will rotate slowly at 5 revolutions per minute. Each time ultraviolet pictures are taken, the satellite will be briefly slowed down by a momentum wheel. A sharp picture will then be ensured by rotating the image on a charge-coupled device to compensate exactly for remaining spin.

Also on board is a pair of sensors for electrons and positive ions intended to observe the interaction of the comet's ionosphere with the solar wind — and particularly to look for turbulence produced by solar flares. Measurements will be coordinated with those from *Sakigake* which will be sitting further "downwind". Although it was launched largely to test the new control and communication subsystems now in use on *Suisei*, it also carried instruments to measure plasma waves and the interplanetary magnetic field.

The launch of *Suisei* is by far the biggest

adventure so far for the Institute of Space and Astronautical Science, the independent academic organization responsible for all Japan's scientific satellites. Preparations have been under way for some

years: a new rocket had to be developed with extra strap-on boosters, a new launch tower built at Uchinoura Space Centre and a new 64-m diameter dish antenna built in Nagano prefecture to pick up the signals. If *Suisei* proves to be a success, a lunar satellite may come next on the list of Japan's ventures into space.

Alun Anderson

University committee review begins

THE promised scrutiny of the British University Grants Committee (UGC) is to be carried out by a committee under the chairmanship of Lord Croom (formerly Sir Douglas Allen), chairman of the British National Oil Corporation; the deputy chairman of the committee is Lord Butterworth, retiring vice-chancellor of the University of Warwick.

The development is important because it is almost certain to lead to a change in the constitution of UGC, founded in 1919 as a buffer between the British government and the publicly supported universities. The need for such a review was most recently advocated by the Jarrett committee on the efficiency of British universities, which reported earlier this year.

The composition of the review commit-

tee is not what British academics would have chosen. Lord Butterworth, made a life peer at the beginning of the year, has been a successful vice-chancellor. The committee also includes Dr Paul Matthews, vice-chancellor of the University of Bath until two years ago, and Mr John Smith, secretary of Imperial College, London. Dr Peter Clarke, chairman of the Scottish Vocational Education Council, was previously principal of Robert Gordon's Institute of Technology at Aberdeen.

The other members of the committee are primarily industrialists, including Sir Alex Alexander, chairman of J. Lyons and Co., Mrs Mark Baker, a director of Thames TV Ltd, Sir Austin Bide, chairman of Glaxo Ltd and Mr Ian Beesley, head of the Prime Minister's Efficiency Unit. □

Soviet science

In the steps of the Ukraine

THE Ukrainian Academy of Sciences, the largest and oldest in the Soviet Union except for the All-Union academy itself, seems rapidly to be becoming a model for the other thirteen republics. During the past few months, the Soviet media have given considerable acclaim to the Ukrainian system of "production centres", semi-pilot factories operating under the academy's aegis. And last month *Pravda* lauded another major Ukrainian innovation — its network of local science centres.

Science centres have been a major feature of Soviet science since the establishment of the Siberian branch of the Soviet Academy of Sciences in 1957. Except for the Ukraine, however, science centres have largely been confined to the Russian republic where, since there is no separate Russian academy, they have come under the All-Union academy.

In spite of official encouragement of science centres as a means of bridging the Soviet Union's endemic gap between research and implementation, the centres have often been criticized for failing to fulfil their allotted tasks. A campaign in the mid-1970s began with the Far Eastern Science Centre and worked its way up to the Siberian branch of the academy before the tide turned.

The Ukrainian network began formally with the establishment, in 1971, of five centres, for the Donetsk, Western, North-Eastern, Dnieper and Southern regions of the Ukraine. By 1980, more than 25 centres

of the whole scientific work-force of the academy was employed in regional centres, spanning marine biology and cryomedicine, high-molecular chemistry and radiophysics. Major developments in the chemical and instrument-building industries have been credited to the centres.

How far the Ukrainian experience could be repeated in other republics, however, is not clear. The Ukrainian SSR is one of the most developed republics of the Union, with large reserves of ores and fossil fuels, as well as much of the best agricultural land. The science centres clearly have had the support of the First Secretary of CPU, V.V. Shcherbytsky, who some years ago publicly urged that Ukrainian scientists should concentrate first and foremost on research needed for the Ukrainian economy. Moreover, the Ukrainian republic is large enough, and has an economy diverse enough, to provide employment for a wide range of scientific talent.

The main problem of the Ukrainian centres as of science in all the non-Russian republics is that of attracting top-flight people. Job prospects in the Soviet Union depend not so much on what, but where, one studies. The brightest students from all republics gravitate towards the top higher education institutions in Moscow and then, almost inevitably, become absorbed into the Moscow-Leningrad-Novosibirsk research network.

WILL BIRD

French nuclear tests

Mystery of *Rainbow Warrior*

LAST month's sinking in New Zealand of *Rainbow Warrior*, flagship of the Greenpeace environmental organization, and the killing of the ship's photographer, have stirred a murky pot of accusation and counter-accusation in France. The French press has taken the scandal to its heart.

One of the more speculative magazines, *VSD*, has even gone so far as to accuse the Elysée palace (the French White House) of having organized the attack to demonstrate French power in the Pacific and determination to continue nuclear weapons testing on Mururoa Atoll; all might have gone well, the theory goes, had not a mole from the British secret service, MI5, buried within its French equivalent, DGSE, tipped off the New Zealand authorities (somewhat too late, it must be assumed). The Elysée will be suing *VSD*,

I was told that Limpet Mines
had no fall-out....



but other speculations implicate KGB, CIA, the home-grown French extreme right and other supposed enemies of equilibrium.

In the more serious French press, a picture is emerging that the two people held and now formally accused in New Zealand are probably members of the French Army seconded to DGSE to survey the activities of Greenpeace. It is suggested that the frogmen who attached two limpet mines to *Rainbow Warrior* left behind their French inflatable dinghy and bubble-free breathing apparatus (stamped "Made in France") and probably escaped in a hired yacht, *Ouvea*, belonged to a separate French group.

The bombers themselves, like *Ouvea*, may have come from New Caledonia, the group of French Pacific islands which is fast becoming President Mitterrand's Algeria — a country peopled by some 91,000 native Kanaks seeking independence and wooed by M. Mitterrand but also 54,000 French colonists who are determined the island shall stay French. These French might have wished to discredit the present government — and hence the trail of evidence left behind, it is suggested.

However, this relatively convenient picture is inconsistent with the fact that

Ouvea was hired (for FF 70,000 cash) through a Paris agency. The hirers were an adventurer French doctor (who specializes in diving medicine, but who left *Ouvea* before the attack and claims he knew nothing of the object of the mission) and three more shadowy figures, since disappeared with the yacht.

The *Rainbow Warrior* crew also believes there was a French "mole" aboard, a woman who sent articles for a "French paper" and one of whose maps of New Zealand waters was later found on *Ouvea* (when the yacht was briefly stopped and then released, to the later chagrin of the New Zealand police). This woman has also disappeared, via, apparently, an archaeological dig in Israel.

President Mitterrand has asked M. Bernard Tricot to carry out an investigation and to inform the public.

But if indeed the French secret service does prove deeply involved, what possible government motive — or perception by junior officials of a government motive — could there have been to bomb *Rainbow Warrior*? The only possibility could be to protect the French nuclear weapons tests on Mururoa, not far from New Caledonia

and Tahiti, which Greenpeace intended to disrupt. The French nuclear arsenal is in continual evolution and even expansion with a postulated introduction of "enhanced radiation weapons" or neutron bombs and a testing campaign of at least another 15 years is planned. Speculation by the French that *Rainbow Warrior* was carrying sophisticated devices for detecting neutron bombs (discounted by Greenpeace, which says the ship carried only a geiger counter and apparatus for relaying pictures to news agencies) suggests that perhaps France was indeed planning a neutron weapon testing programme.

But whatever the merits of this speculation and of an attack on Greenpeace as a counter-measure, the opposition of South Pacific states to French testing, at the rate of 60 weapons between 1977 and 1984, is now liable to increase, since the signing earlier this month by seven nations of a declaration that the South Pacific should be a "nuclear free zone". The treaty, which forbids signatories to build or test nuclear weapons, will be ratified by the end of the year, Australian Prime Minister Bob Hawke has predicted, and though powerless to prevent French testing in the region it will certainly increase French political isolation. The *Rainbow Warrior* affair can only have done the same.

Robert Walgate

French mistrusted on Mururoa

MURUROA Atoll, according to New Zealand and Australian scientists who visited the French nuclear weapons testing site in October 1983, is likely to release its accumulated load of debris from underground explosions to the Pacific "within 500–1,000 years".

At present, they say, emissions from the island and consequent fallout in the South Pacific region are having a negligible effect on health and they do not suggest that even the likely release of the contained radiation within a few centuries will be "consequential". But the scientists' evident suspicion of French calculations about the security of the site is symptomatic of the South Pacific's distaste for what the French are doing with "their" atoll.

For example, "French information on venting is very qualitative", the South Pacific scientists say in their report, "and there is no reason to believe their figure that more than 99 per cent of the radioactivity is retained in vitrified material". And any "implicit impression" that venting is barely detectable is "almost certainly false".

On underground containment, the report indicates that the French tests are "probably" 700–1,200 m below the bottom of the lagoon, in the volcanic base of the atoll, which is surmounted by 180–500 m thickness of limestone. But the limestones contain some highly porous sections and the volcanics are fractured for 400 m

around each explosion. "The volcanics in their virgin state offer a poor to moderate geochemical barrier and a moderate to good hydrological barrier. "The testing programme is reducing the effectiveness of both", the report says. Moreover, "the claim that the transition zone [between the volcanics and the porous limestone] acts as a barrier to long-term leakage can, on the basis of geological evidence, be discounted".

Thus there will be leakage within 500–1,000 years, in which transuranic elements without a particular geochemical affinity (such as neptunium-237) may be expected to be leached out. "It would be surprising if this leakage were consequential", the report says, but such scientific assurances, according to an Australian government spokesman last week, have made very little impression on public opinion. Rational or irrational, the public view is that far more atomic weapons have now been exploded in the South Pacific than in the north (the public thinks of Hiroshima and Nagasaki) and that it is simply high time the bombing stopped.

But France will not stop, which may, in the long run, lead to a search for a new site. A deal with French Africa to use the Sahara (from which the testers decamped to Mururoa in 1966), or a deal with the United States to use its own sites, emerge as the only faint possibilities.

Robert Walgate

Mt Wilson Observatory

SIR—We have recently returned from what seems all too likely to be our last observing run as guests of the Carnegie Institution with the 100-inch telescope at Mt Wilson, California. We had 14 consecutive nights on the telescope. All of them were clear, the only cloud that we saw during that whole time was a little cirrus, which did not impede us at all, on one night. Star image diameters near one second of arc were the norm, and on several occasions we enjoyed "seeing" much better than that — the remarkable seeing for which Mt Wilson is renowned.

To experience such enviable observing conditions for such a sustained period on our final observing run heightens the irony of Mt Wilson Observatory being closed down ostensibly because of the deficiencies of the site. The 100-inch was shut down finally, as far as its owner the Carnegie Institution is concerned, on 26 June 1985.

Like every one of the major instruments on Mt Wilson, the 100-inch has played a role of extraordinary distinction and significance in the progress of astronomy throughout much of this century. But it should not now be allowed to rest on its laurels. The same telescope that served Hale and Adams, Hubble and Humason, Baade, Sandage and countless others so well in the past is still in the same class as the very best of the newer telescopes, and behind its now-closed shutters it sits ready and waiting to resume the battle with the pressing problems of present-day astronomy. In this connection it is of interest to recall that observers at the 100-inch, casually viewing Saturn, were well aware of the "gramophone-record" appearance of the rings¹ before *Voyager* drew attention to that structure; but an experienced planetary observer who observed Saturn under the best conditions at the 200-inch Palomar telescope specifically asserted² that no subdivisions exist in the rings.

The very short time-scale upon which the announcement (made barely a year ago) of the closure of Mt Wilson is being implemented has made it impossible for any other organization yet to assume financial and operational responsibility for the observatory. Although the 100-inch telescope is the first to close, the 60-inch and the solar telescopes will soon follow it if they too are not taken over by other operators. The formidable researches that are being undertaken with those instruments, all of them long-term projects that would suffer severely from even a temporary interruption, are described with extraordinary honesty in the current annual reports of both the president of the Carnegie Institution and the director of the Mt Wilson & Las Campanas Observatories. The juxtaposition in those reports of the current achievements of Mt Wilson Observatory and the closure of the self-

same place leaves the reader altogether incredulous.

The Athay Committee, which was set up at the request of the director of Mt Wilson to advise on the future of the observatory, rightly refers to the "anguish" of astronomers around the world at the closure of such a distinguished observatory. The committee's report makes a strong case — actually not a difficult thing to do — for the retention of Mt Wilson in operation, and includes a recommendation that Carnegie should at least continue to run the observatory for a reasonable period until an orderly takeover by another operator can be arranged. Unfortunately, the Carnegie Institution has apparently not been able to counter reasoned argument with reasoned argument, but instead has chosen to respond with ever fiercer press releases that appear to constitute its only acknowledgement of the receipt of the report which it requested. Its position seems analogous to that of St Paul on the Damascus road before his conversion³; we devoutly hope that the parallel is completed and/or that some other institution will soon be found or set up to maintain and even enhance this great observatory. Long live Mt Wilson!

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- 1 Livingston, W C *Sky Telescope* 49, 207 (1985)
- 2 Kuiper, G P *Trans int astr Un* 9, 255 (1957)
- 3 Acts, ch 9, vv 1ff

Soviet tests

SIR—If your reporter meant to imply in his report on the American Association for the Advancement of Science (AAAS) meeting in Los Angeles¹, that I accused the US government of lying on the question of Soviet compliance with the Yield Threshold Test Ban Treaty (YTTBT), I must take exception. I personally have no way of knowing for certain whether the assertions by some representatives of the US government^{2,4} that the Soviet Union has tested over the treaty threshold of 150 kton are accurate, even less do I have any grounds for implying intent to deceive on the part of those representatives.

What was pointed out at the AAAS meeting is that the techniques for calculating unbiased estimates of the yield of underground nuclear tests have been well understood for over a decade^{5,6}. The most recent published work that I am aware of⁷ concludes that within the resolution of the seismic data and analysis techniques used, the Soviet Union has not significantly exceeded the 150 kton limit. Unlike most questions of Soviet treaty compliance, this work does not depend upon classified data or analysis techniques. If the US Depart-

ment of Defense (DOD) has reason to believe that this work is in error, it has a right and an obligation to demonstrate that in the open literature.

However, recent reports indicate^{4,8} that work within DOD supports the finding that the Soviet Union is complying with the terms of YTTBT. If these reports are correct, DOD should make the information public. Surely no-one can have forgotten the bitter lessons of the past 20 years; in a free democratic society, the bond of trust between the government and the people is also a vital component of national security. Moreover it is unthinkable that responsible people would let false pride over a small technical error contribute to making matters any worse than they already are between the two great nuclear powers.

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- 1 Beardsley, T *Nature* 315, 445 (1985)
- 2 US Dept of State, Security and Arms Control *The Search for a More Stable Peace*, 57 (Bureau of Public Affairs, Washington, DC, 1983)
- 3 Alewine & Bache *EOS Trans Am Geophys Un* 64, 193 (1983)
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- 5 Everden & Filson *J geophys Res* 76, 3303-8 (1971)
- 6 Marshall *et al Geophys J R astr. Soc* 57, 609-638 (1979)
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- 8 *Science* 228, 1072 (1985)

Prehistoric calendar

SIR—Correspondence has recently revealed a mistake of 1° in the azimuth given for the equinoctial sunset notch at the prehistoric site at Brainport Bay, Argyll (*Nature* 314, 158; 1985). The reduction of six solar observations taken at the Oak Bank stone backsight gave an average azimuth of the notch of 99.46° west of true north and its declination of +0° 8' was calculated from this. Somehow this azimuth became a true bearing of 261° 32.5' in the text and in Fig. 4, whereas it should of course be 260° 32.5'. A fresh reduction of the same observations by a different method gives the same azimuth and a declination of +0° 9.6'.

On 21 March 1985, about 26 hours after the astronomical equinox, the setting Sun was observed from the Oak Bank rock carving until it was about a solar diameter above the horizon, where it was photographed; at that point it disappeared into a bank of cloud but the disk was clearly heading directly into the notch. This of course confirms the calculations and also the inference drawn from them that we have at Brainport Bay a precise and still functioning equinoctial alignment.

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The science of sea dumping

SIR—In their letter on the science of sea dumping (*Nature* 11 July, p 100), Alan Reddish and Steve Cousins state that "scientific modelling should clearly specify the model in terms of the underlying theoretical assumptions". We agree, which is why the forthcoming Nuclear Energy Agency (NEA) report on the review of the continued suitability of the north-east Atlantic dump site¹ contains over 60 pages of description of the models developed by the Ministry of Agriculture, Fisheries and Food (MAFF) and the National Radiological Protection Board (NRPB) to assess the radiological impact of sea dumping of radioactive waste on both humans and marine organisms.

Clearly, Professor Holliday and his team² could not include all this detail in their report and there was no need since it is to be published elsewhere. However, the full description was provided to them, to the NEA expert group for the dump site review and to the panel of scientists carrying out the London Dumping Convention review.

Reddish and Cousins also express concern about experimental validation of models, but they fail to realize the difficulties involved and the extent to which field measurements have in any case been used. Direct validation of the whole suite of models is simply not possible because of the large time and space scales involved and the very low radionuclide concentrations. What can be done is to validate those parts of the models that have most effect on predicted radiological impact.

Studies of model sensitivity show clearly that it is the interactions between radionuclides and suspended and seabed sediments that most influence doses. This part of the model has been validated¹, using data on naturally occurring radionuclides in the ocean. Data from the earlier dumping site in the English Channel cannot be used for direct validation because the site is coastal while the models deal with dumping in the deep ocean and because monitoring has failed to detect any radioactivity that can be traced to this site. Irish Sea data are also coastal and so could not be used for direct validation, but they can be, and were, used in fixing some model parameters, particularly the factors by which coastal water organisms concentrate radionuclides.

One method of telling whether the results of a particular modelling approach are robust is to tackle the problem in a conceptually different way. The basic model used to estimate dose to man was first of all to predict the distribution of radionuclides in space and time, using an oceanographic model and then to calculate the expected concentrations of radionuclides in sea foods by the application factors.

The concentration factors anticipate the concentration of radionuclides in surface and mid-water marine organisms, relative to the ambient water, as a result of the accumulation of radionuclides from all sources including the ingestion of food. The values used are, for the most part, derived from direct observations. The sea foods eaten by man are all taken in coastal and surface waters to depths of up to 1,500 m.

No sustained food chains from the deep sea (> 4 km) to man are known but, as a safety measure, calculations were also made of the dose to man that could arise if fish living at the dump site itself, close to the bottom, were directly consumed by man. All these calculations indicate very low expected dose rates to man at daily consumption rates of considerable quantities of sea food.

The conceptually-different approach used in the sensitivity analysis was to estimate the dose to man that might arise if, by some chance, contaminated organisms within the dump site were consumed by organisms from outside the area, resulting in radionuclides eventually being transferred to man at concentrations that would not be predictable from the ambient water concentrations, and over a much shorter time scale.

The results of making a number of calculations, based on various ideas as to how such a transfer of radionuclides could in theory be effected, led to the conclusion that even if such occurrences do take place, the resulting dose to man would still be exceedingly low.

There is often a common misconception as to what, exactly, models are attempting to achieve. In this case it is an attempt to estimate the dose to man, and the impact on the deep-sea fauna, as a consequence of disposing packaged radioactive waste into the deep sea. The models used do not aim to represent in detail all known geochemical, oceanographic and biological processes. What they do attempt is a distillation of the major processes and parameters that would be primarily concerned in estimating the dose to man and, by the application of sensitivity analyses model-to-model comparisons, and a verification of major parameters by environmental observation, produce sufficient information for decisions to be made.

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¹ NEA Review of the Continued Suitability of the Dumping Site for Radioactive Waste in the North East Atlantic (Nuclear Energy Agency, OECD, Paris, in the press)

² FGT Holliday Report of the Independent Review of Disposal of Radioactive Waste in the Northeast Atlantic (HMSO, London, 1984)

Pain and animals

SIR—R. Sharpe's letter (*Nature* 25 July, p. 290) contains a sentence beginning "Since it is impossible to devise a method of measuring pain in animals it follows that...". If this premise was true, it would indeed follow that regulations against cruelty to animals would depend on doubtful subjective judgements. The problem is not how to assess an animal grossly disturbed by some acute injury which no humane human would allow to continue. Veterinarians and scientists are faced with the much more difficult problem of judging animals with a chronic disorder such as arthritis.

A number of groups have proposed solutions and in particular the International Association for the Study of Pain has published its obligatory guidelines (*Pain* 16, 109–110; 1983). They require a two-stage investigation. First the animal in question is compared with normal animals by measuring quantitatively its behaviour such as movement, grooming, sleep etc. and by measuring its physiology such as EEG, hormone levels, autonomic state and so on. The animal is then treated with a relevant analgesic procedure and its behaviour and physiology are again measured. The first measure describes the abnormality of the animal in objective terms and the second measures the degree of abnormality attributable to pain. It is as crucial for animals as for babies that we should use every available skill to measure their pain in spite of the fact that we cannot speak to them.

P.D. WALL

(Managing editor, *Pain*)

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Improving crops

SIR—Close to this town lives a tribe of Indians who practise a clearly defined method for improving their crops, particularly maize. Every family plants its own seed, avoiding contamination with alien germplasm. When they find an outstanding example, they do not use it for food or seed; they give it as a present to a friend, who is not supposed to eat it or use it in any other way, but to plant it. The whole action is treated as a religious ceremony or social convention, presumably part of a system ensuring the tribe's continuity. The exchange involves a large part of the community, and is made in all directions.

The obvious objective is to increase the frequency of favourable genes with additive actions, by selecting within a mildly inbred population, in which new forms with desirable characteristics are introduced periodically.

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Selenium not for dumping

California is up in arms because of selenium in some drinking water. Does it not know that selenium is also an essential dietary supplement?

WHY do so many land animals depend so crucially on inorganic substances predominantly present only in sea water, selenium for example? The question has most recently been provoked by the fuss, in California, about the discovery last year of selenium in the water stored in the Kesterson reservoir. Officials of the city of Monterey have since refused to allow that supposedly toxic fluid to be allowed to return to the Pacific Ocean. In their ignorance, they have overlooked a question that is not merely of practical importance (how do we keep our people healthy?) but one that has profound significance for people everywhere — where did we come from?

This is how the tale goes. Life began in the oceans or shallow seas. The primitive enzyme systems depended on inorganic ions: (Physiologists have often remarked on the resemblance between body fluids and sea water.) But some of these vital inorganic substances are needed in only small amounts. These are the nutritionally essential trace minerals, such as iodine, molybdenum, manganese and zinc. When living organisms emerged from the oceans, animals and plants that became land-dwellers had to find soil that supplied these minerals. Iodine is uncommon except near the oceans: Plants do not need it, but goitre became an endemic disease of animals in inland areas lacking iodine.

Geological variations in the Earth's crust, glaciation during the great Ice Age, the effects of vulcanism and the results of erosion and leaching are some of the factors that decide which parts of the Earth's surface are either deficient or oversupplied with trace minerals.

In some parts of the world, most conspicuously in Australia, the soil is so deficient in certain trace minerals such as zinc and copper that plants will not grow. Elsewhere, soil contains a toxic excess of some minerals. One of these is selenium, which was known for many years only as being poisonous, even carcinogenic. It came into prominence because certain plants such as *Astragalus* (locoweed) concentrate it from high-selenium soils so that horses that eat *Astragalus* may become poisoned, or "locoed" ("blind staggers"). In the 1930s, Franke and his co-workers reported effects of "toxic wheat" grown in South Dakota on high-selenium soil.

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Feeding toxic wheat or selenium to hens produced deformed embryos in their eggs with eyes and beaks missing, and with distorted wings and feet¹. No such injuries were detected in human beings; the only bad effects noted were dental caries.

The bad reputation of selenium was abruptly changed in 1957, when it was found in laboratory studies to be a nutritionally essential trace element for rats, chickens and pigs^{2,4}. This news was followed with startling rapidity by reports of selenium deficiency in farm animals all over the world, especially in New Zealand. Selenium provided one of the most dramatic illustrations of the rule of Paracelsus that "the dose alone determines the poison", a rule that seems to have been forgotten by most of us.

Among the many studies of the toxicity of selenium were some carried out by the US Food and Drug Administration (FDA) which led to the conclusion that high levels of selenium are carcinogenic⁵. Since official thinking does not permit the extension of Paracelsus' rule to carcinogens, FDA agonized for 15 years before it allowed selenium to be added to the diet of farm animals.

Ironically, selenium had meanwhile emerged as an anticarcinogen and as a component of an important enzyme, glutathione peroxidase, an enzyme important in the metabolism of injurious hydroperoxides⁶.

Now move to China, where multiple myocarditis, "Keshan disease", is found in children in populations with low levels of blood selenium living in low-selenium areas. Epidemiological studies show an inverse correlation between blood selenium and age-adjusted total cancer death rates and liver cancer incidence. In areas with high selenium levels, there is significantly lower mortality from cancer of the oesophagus and stomach. As a public health measure, a programme has been started of spraying grain crops with sodium selenite solution⁷. The difficulty may be that both the population and the food supply may be less mobile than in the United States.

In California (where anything can happen, and usually does), selenium leaching from the coastal foothills of central California (perhaps a result of acid rain) got into irrigation water and became concentrated in drainage reservoirs. Aquatic birds, coots and wild duck, living in the Kesterson reservoir, re-enacted in

1984 the 1930 drama of the South Dakota chickens, producing deformed offspring. Environmentalists sprang into action, saying that selenium would probably cause deformed human embryos. The headlines flared. Officials in Monterey acted. A former head of California Water Resources Control Board spoke of the "Kesterson nightmare", and predicted⁸ "another year's crop of what may very well be contaminated produce".

In reality, the "contaminated produce" is probably helping to protect consumers against cancer^{9,10}. The permissible level of selenium in drinking water, set by the Environmental Protection Agency (EPA) at 10 parts per thousand million, should probably be raised to 50 to 100 parts per thousand million as a public health measure.

Until FDA gave approval for selenium as an animal feed additive, farmers literally had to return to the ocean, the original source of selenium for animals, to obtain selenium for their livestock. This was done by feeding fishmeal, the selenium in which was natural and therefore legal. Selenium has another role in ocean fish; it prevents the injurious action of mercury that is naturally present in seawater and is added to the ocean by volcanic discharge and run-off from coastal soils.

Various hearings on the disposition of the Kesterson water have brought forth recommendations ranging from stopping agriculture in the vicinity of Kesterson to skimming off the surface soil and burying it. In none of the hearings has the essential nature of selenium, or the fact that selenium is a natural resource that tends to be depleted and lost, been discussed or even mentioned. Nor have tolerable levels of intake been suggested. The objective seems to be to treat selenium as a toxicant that must be sequestered, yet much of the land in California and more in Oregon, is deficient in selenium. Shall we have to go back to the sea?

Thomas Jukes

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Sodium pump

Birthday present for digitalis

from D.G. Allen, D.A. Eisner and S.C. Wray

EXACTLY two hundred years ago, William Withering published his monograph on the therapeutic use of the foxglove *Digitalis purpurea* in congestive heart failure, then known as dropsy¹. Digitalis is known to bind to the (Na⁺ + K⁺) ATPase, the enzyme that acts as the Na⁺/K⁺ pump in the membrane of cells, and in consequence inhibits the extrusion of Na⁺ and the inward movement of K⁺ ions². The publication, elsewhere in this issue, of the amino-acid sequence of the α -subunit of the (Na⁺ + K⁺) ATPase^{3,4} makes a fitting celebration of the bicentenary. But knowing more about the structure of the Na⁺/K⁺ pump and the way in which cardiac glycosides may affect it should not be allowed to obscure the uncomfortable fact that inhibition of Na⁺ and K⁺ movements may not be the sole or even the most important therapeutic action of the cardiac glycosides.

Although cardiac glycosides, such as digitalis and ouabain, are now among the most commonly used cardiac drugs, many aspects of their mechanism of action and their clinical use are still controversial. Three questions of pharmacological interest are currently claiming attention: how the cardiac glycosides increase the force of heart-muscle contraction (positive inotropy); whether there are naturally occurring cardiac glycosides in human plasma; and whether the cardiac glycosides are equally valuable in all types of heart failure. Recent work has suggested that these issues may be related and may lead to a better understanding of the way in which digitalis works.

The conventional view of the positive inotropic mechanism is shown in the figure (refs 5,6). Briefly, digitalis inhibits the Na⁺/K⁺ pump, leading to an elevation in the intracellular Na⁺ concentration, which acts on a membrane Na⁺/Ca²⁺ exchange to increase the intracellular calcium concentration; this increases the Ca²⁺ content of the sarcoplasmic reticulum and leads to release of Ca²⁺ during systole. There is good experimental support for the increase in the intracellular Ca²⁺ concentration, which represents the final stage of this process⁷. Support for this scheme comes from the fact that other manipulations that inhibit the Na⁺/K⁺ pump also increase the force of contraction. Under these conditions, there is a close relationship between internal Na⁺ concentration and force⁸. This hypothesis has, however, been criticized on two grounds⁹. First the plasma concentration of cardiac glycosides in patients is about 10 nM whereas Na⁺/K⁺ pump inhibition, at least *in vitro*, is only seen at a concentration of about 100 nM (ref. 10). Second, low

concentrations of cardiac glycosides have been reported to stimulate rather than inhibit the Na⁺/K⁺ pump in various isolated preparations, as shown by measuring either ATPase activity¹⁰ or internal Na⁺ concentration¹¹.

Interpretation is complicated because it has been suggested that the apparent pump stimulation is secondary to release of catecholamines from nerve endings¹². This criticism cannot, however, be levelled against the demonstration that the isolated ATPase is stimulated by cardiac glycosides¹³. These experiments have also shown that there is a maintained increase of activity that is not further increased by low doses of glycosides if the ATPase is first washed with a salt solution. This suggests that the stimulation might result from displacement of an endogenous

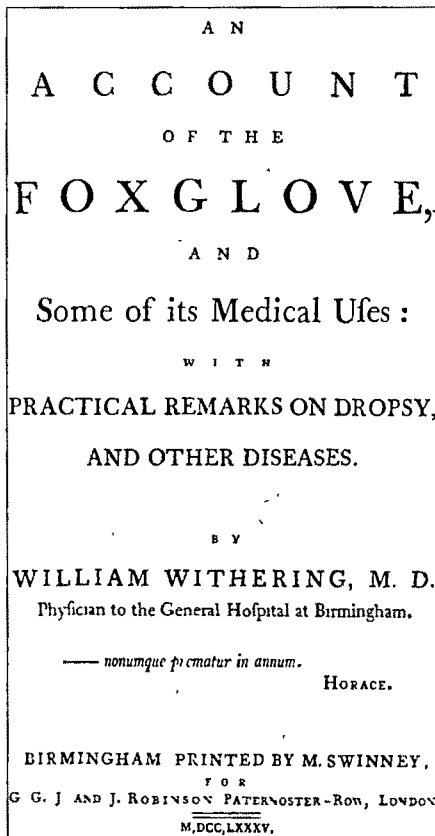
actually inhibiting the Na⁺/K⁺ pump. The major unresolved question of the 'non-inhibition' hypotheses is how glycoside binding can increase force. Possibly binding may interfere with Ca²⁺ bound to the surface membrane¹⁵. Another intriguing experimental finding is that intracellular injection of low concentrations of glycosides can lead to an inotropic effect that is independent of binding to the extracellular Na⁺ pump site¹⁶. Despite these problems with the Na⁺/K⁺ inhibition hypothesis, however, no clear alternative has yet been proposed.

There is much interest in the possibility that the cardiac glycoside receptor on the Na⁺/K⁺ pump was 'designed' to bind an endogenous glycoside-like substance present in human plasma. As Na⁺/K⁺ pumps are found in a wide variety of tissues, such an endogenous substance would cause, amongst other effects, increase in cardiac contractility, constriction of blood vessels and an increase in Na⁺ excretion by the kidney. Endogenous Na⁺/K⁺ pump inhibitors have been found in toad skin¹⁷ and rat hypothalamus¹⁸, and a substance that inhibits the Na⁺/K⁺ pump has recently been isolated from the blood of hypertensive patients¹⁹. This is particularly interesting because it may be a cause of essential hypertension. Release of this substance in response to a plasma Na⁺ load could increase Na⁺ excretion by the kidney; possibly it is the elusive natriuretic hormone²⁰. This substance should, however, also inhibit the Na⁺/K⁺ pump in vascular smooth muscle, so producing vasoconstriction and consequent increase in blood pressure.

Information about the structure of the Na⁺/K⁺ pump may help us to understand how it is affected by glycoside binding. The sequence data allow us to identify the parts of the molecule that lie within the membrane and those that protrude into the extracellular and intracellular spaces. The ATP binding site and the phosphorylation site are also revealed, and preliminary suggestions can be made about the position of the glycoside binding site. It is particularly instructive in this regard to compare the (Na⁺ + K⁺) ATPase sequence with that of the Ca²⁺-ATPase, also published in this issue²¹. Ca²⁺-ATPase does not bind glycosides and the molecule lacks the sequence that Schull *et al.*³ suggest is the ouabain binding site on the (Na⁺ + K⁺) ATPase.

One of the first questions is how glycoside binding leads to inhibition of the ATPase and to the movement of ions, but more detailed knowledge of the binding site and possible interactions with other regions of the molecule may suggest further consequences of glycoside binding that could underlie the therapeutic effects. For example, glycoside binding might lead to a conformational change in the molecule, thus affecting Ca²⁺-binding in the surrounding membrane.

The amino-acid sequences and structural information so far published show



inhibitor (see below). One problem with this hypothesis is that simply displacing one inhibitor by another at the same site should not result in a stimulation of the pump.

In this context, it is interesting that recent measurements of ouabain binding to membranes have shown two classes of binding sites^{11,14}. The lower affinity one may be the conventional site at which the Na⁺/K⁺ pump is inhibited, whereas the high affinity one might provide an alternative positive inotropic mechanism without

that the Na^+/K^+ pumps from the electroplax organ of *Torpedo californica*⁴ and the kidney of sheep⁵ are strikingly similar. Both α -subunits have 1021 or 1022 amino acids, about 87% of which are homologous, and there are runs of 128 and 108 amino acids that are identical. Six regions of 22–29 hydrophobic residues are probably big enough to span the membrane and the intracellular regions of the molecule can be identified from the locations of the ATP binding site and the phosphorylated site. It is assumed that the glycoside binding site(s) lie on one of the three regions that protrude into the extracellular space. Assuming the glycoside binding site can be unequivocally identified in future, it will be of considerable interest to see to what extent the glycoside binding sites are conserved in pumps from different tissues and species and, in particular, to compare species that have very different sensitivities to cardiac glycosides. Homologies between this and other ion-pumping ATPases^{1,2,21} are substantially lower but offer the intriguing possibility of identifying the crucial amino-acid sequences for common functions, such as ATP binding and energy transduction.

At present cardiac glycosides are used clinically to increase cardiac output in two major conditions. In atrial fibrillation, where the increase in heart rate renders the heart inefficient as a pump, the glycosides, through their action on the vagus nerve, slow the heart and so increase its output. They are also used to treat heart failure, where the rate is normal, but the efficacy of digitalis under these conditions is controversial: Although digitalis treatment can produce a small, short-term benefit²², controlled trials have found little evidence of benefit from maintained digitalis therapy. This may, of course, simply mean that the initial treatment allowed

the heart to recover. Alternatively, prolonged exposure to glycosides could result in the synthesis of more Na^+/K^+ pumps²³, so compensating for those inhibited by the glycosides. If there is an endogenous glycoside-like substance that competes with the therapeutic glycosides for the inhibitory site on the sodium pump, it will be necessary to monitor its concentration during treatment before we will understand the therapeutic role of glycosides.

Whereas, in isolated preparations, large doses of glycosides lead to a substantial inotropic effect that can be accounted for by the mechanism shown in the figure, at the low doses used therapeutically the inotropic effect is much less marked. Even so, it leads to a small but worthwhile clinical improvement. The magnitude of this low dose effect may well depend on the presence of endogenous factors that compete for the same binding site. □

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Astrochemistry

Chemistry in dynamically evolving interstellar clouds

from David A. Williams

RECENT observations of interstellar molecular clouds have shown that they have a great diversity in density, temperature and chemical state. The study of these clouds has become important in astronomy because it is generally believed that they are the progenitors of stars. In an important theoretical contribution, Tarafdar *et al.*¹ have described a framework within which the diversity of the clouds may be understood. They suggest that the observed variety of conditions and molecular state is a natural outcome of the evolution of the clouds, as they contract under gravity from low density atomic gas to high density molecular material.

Chemistry in interstellar clouds is now a well established subject² and the mathematical modelling of extensive reaction networks has been investigated by many authors during the last fifteen years. These studies have shown that whereas low density clouds are generally in a chemical steady state, time for denser clouds to approach a steady state — typically 30 million years (ref. 3) — is probably greater than the age of the clouds. It is unlikely, therefore, that dense clouds have achieved a chemical steady state, in spite of the apparent success of some steady state models⁴. A time-dependent approach is necessary, especially if one assumes that molecules in the gas collide and stick to dust grains and are not returned to the gas. If this is the case, a

steady state can never be achieved. The time required before this process seriously depletes molecules from the gas is about $10^3/n$ years, where n is the density of hydrogen atoms per cubic centimetre. This time is obviously quite short when the density is high, say $n > 10^4 \text{ cm}^{-3}$. Time-dependent models of the chemistry of static clouds^{5,6}, however, also fall short in some significant respects when compared with the observations, and the models clearly require further development.

The latest refinement to be included in the models depends on the recognition of the dynamical character of interstellar clouds. The spectral line widths observed from the clouds are several km s^{-1} , much broader than would be expected (about 0.1 km s^{-1}) for gas at rest at the temperatures of interstellar clouds, indicating there must be considerable motion in the gas. Therefore, turbulence, low velocity shocks, expansion or contractions must be driving the gas to supersonic velocities. For example, J. E. Dyson, T. W. Hartquist and D. A. Williams (ref. 6 and in preparation) have developed a model of giant molecular clouds in which continuous low-mass star formation creates fast winds that drive the bulk motions and erode dense clumps of gas⁷, maintaining the chemistry permanently out of steady state.

In an alternative approach, Tarafdar *et al.*¹ have considered an isolated, spherical, initially low density cloud. For gravita-

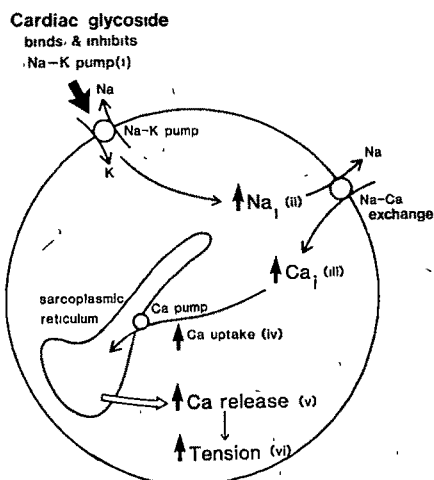
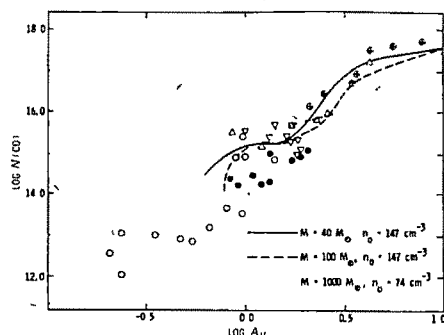


Diagram of a cardiac muscle cell, showing the conventional view of the positive inotropic action of the cardiac glycosides. Binding inhibits the Na^+/K^+ pump (i), elevating the intracellular Na^+ concentration (Na^+ (ii)) and thence intracellular Ca^{2+} (Ca^{2+} (iii)). This leads to an increase in the Ca^{2+} content of the sarcoplasmic reticulum (iv), with increased release of Ca^{2+} (v) and increase in tension (vi).

tional collapse of the cloud to occur, its thermal and turbulent pressure must be insufficient to hold up the cloud against gravity. The temperature structure of a cloud is therefore of paramount importance and Tarafdar *et al.*¹ have carefully determined the temperature profile of low density clouds. They have shown that such clouds are indeed gravitationally unstable and will collapse, without the need to involve a trigger of shocks or thermal instabilities, as had been suggested by earlier workers. They consider that this collapse explains the observed large line widths. They follow the collapse numerically, using a hydrodynamic computer code developed by Bodenheimer², and simultaneously follow the evolving chemistry in the gas, using a restricted version of the reaction network described by Prasad and Huntress³. In addition, the description indicates the loss of molecules by sticking to grains. Their results show that the extinction at the cloud centre increases as the collapse proceeds, and the molecules are freed from the major loss caused by photodissociation, with the result that their abundance rapidly build up.

The models of Tarafdar *et al.*¹ provide some very interesting conclusions. In all cases, regardless of the starting conditions, central densities attain very high values ($n > 10^6 \text{ cm}^{-3}$) within a few million years. A few percent of the cloud mass is in the form of this high density clump at the cloud centre. If these clumps proceed to form stars, a plausible rate of star formation for the Galaxy can be derived.

The chemical condition of the gas is also fully explored. Because the gas spends much of its time in a low density state, the predicted molecular abundances are dramatically affected. The excellent success of the model in describing the chemistry may be judged from the figure, which shows how the fraction of gas in the form of CO varies with extinction, A_v , along the cloud diameter (in this model, extinction is merely another measure of elapsed time in the contraction). A high C-atom abundance is also derived from these models. Observations of other atoms and molecules are also plausibly encompassed in this description. Thus, the observed differences are merely snapshots at different



Comparison of theoretical and observed variation in $N(\text{CO})$ with A_v . Modified from ref. 2, where references to the original data sets shown by the various symbols are given.

instants during the continuing collapse of clouds from a diffuse state to some dense state, from which — presumably — stars are formed. The initial diffuse clouds are all predicted to be fairly similar.

Several questions deserves further study: for example, will the high C-atom abundance drive the formation of unacceptably high hydrocarbon densities? Do clouds really evolve as rapidly as suggested? If stars do form, will the cloud remnant be entirely disrupted? Is all high density material in the form of transient clumps? The answers to such questions will surely be forthcoming. Tarafdar and colleagues have opened up a very

promising avenue in the study of the interstellar medium and star formation. □

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Cellular immunology

Suppressor T cells in the network

from N. A. Mitchison

MOST of the elements of any complex control system look inwards at one another — in the immune response most lymphocytes interact with one another rather than with the outside world, just as neurones do in the nervous system. Within the regulatory compartment of the immune system the major components are helper and suppressor T cells (Th and Ts cells) which control the activity of the cytolytic T cells and antibody-producing B cells of the effector compartment. Helper T cells act directly on these effector cells but suppressor T cells usually act indirectly, via inhibition of helper T cells. It is this inhibitory interaction between suppressor and helper T cells that is analysed by two papers on pages 738 and 741 of this issue^{1,2}.

The question asked is who recognizes whom: do Ts recognize Th, or is it the other way round? This sounds simple enough, but it is made complicated by the dual nature of the receptor that T cells bear. It recognizes both antigen and a MHC (major histocompatibility complex) molecule, a property valuable to T cells because it prevents them from interacting with free antigen and ensures that they react only when it contacts other cells.

This obviously matters for cells like T cells, which operate only at short range, and is not important for B cells which secrete long-range antibody molecules. Tada has termed interactions of T cells with other cells as both 'linked' and 'cognate'. Linkage occurs via an antigen bridge connecting the antigen-binding parts of receptors on the two cells, and cognate binding of the T-cell receptor to an MHC molecule occurs on the partner cell ('cognate' means that receptors can recognize the MHC molecule as a result of repertoire selection in the thymus). In the Th-Ts interaction the 'link' must be symmetrical, that is, receptors on both cells bind to separate parts of a single antigenic structure. The direction of recognition is determined by the cognate part of the in-

teraction: if the receptor on the Th cell binds to an MHC molecule on the Ts cell, then it is the Th cell that is doing the recognizing, or vice versa.

An additional possibility is that receptor idotype (a structure on the variable part of the receptor) can substitute for antigen, in which case the linkage involves simply one receptor binding directly to another. Note that this type of binding is entirely symmetrical, because in such an interaction one cannot in principle distinguish between the idiotype structure and the receptor that binds to it.

During the past five years two substantial studies seemed to have answered the 'who-recognizes-whom' question^{3,4}: both reached the conclusion that the Ts cell contributes an MHC molecule and is therefore the partner which is recognized. It releases this molecule as a single chain, linked by a disulphide bridge to a second antigen-binding chain, which in turn establishes an antigen bridge with the Th cell. There was still some uncertainty as to whether the Th cell used its standard receptor (the 'Ti' receptor, which it uses to interact with antigen-presenting cells and B cells) for this purpose. That is where the I-J molecule, or determinant, comes in. Klein *et al.*³ argue that the Th cell does use its standard receptor, and that the I-J structure which distinguishes Ts cells from other lymphocytes is simply a modification of an MHC class II molecule — perhaps a set of determinants unique to an isolated (monomeric) MHC chain as suggested by Bodmer⁴. Despite these small differences, at that time there was general agreement about the major conclusion, and a substantial series of experiments seemed to confirm predictions made by the 'Th-recognizes-Ts' hypothesis. In addition, the hypothesis seemed to fit with the growing evidence for 'veto' cells, antigen-presenting cells within the thymus which also inactivate any Th cells that recognizes them.

These suggestions are all contradicted by the experiments reported in this issue^{1,2}, which show quite clearly that the recognition properties of T_s cells are determined not by the MHC molecules which they bear, but by selection of their receptor repertoire in the thymus. Thus, lymphocytes from allogenic chimaeras can make the soluble factor with suppressor activity (T_sF) of host type even though they do not bear host MHC molecules (they can also make donor-type T_sF, presumably because both donor and host MHC molecules are present during repertoire selection in the thymus). The conclusion is that what was previously regarded as an MHC molecule should now be reinterpreted as an anti-idiotypic which binds to that part of the T_h cell receptor that recognizes MHC molecules.

Much of the data of Klein *et al.* do not contradict this view (see ref. 3 for review). All that is needed is the substitution of an anti-idiotypic of the required type wherever an MHC molecule was identified previously as a restriction element.

Note that the concern expressed by Urcz *et al.*² about expression of I-J on macrophages must be misplaced: host macrophages are presumably needed to elicit idiotypes characteristic of the host MHC-binding site on T_h cells that in turn permit T_sF to express anti-host idiotypic binding activity.

Other possibilities exist but they all seem to involve discarding even more experimental data. Meanwhile we are still waiting for crucial molecular information, particularly about the difference between the mouse strains used to raise anti-I-J antibodies, and about the relative molecular mass 33,000 peptide that is precipitated by these antibodies⁵. If the interpretation offered in the two new reports^{1,2} is correct, the peptide must be part of the T_s cell receptor for antigen + MHC.

There is nothing new for cellular immunology in having to reinterpret quite large chunks of data; that has already happened, for instance, during the elucidation of *IR* genes. No doubt it will do T_s cells no good at a time when they are, for other reasons, running into heavy weather⁶. Nevertheless, these important papers from Japan must be regarded as a major achievement for the idiotypic network theory, and they will strengthen the view already current⁶ that T cells are even more strongly subject to network control than are B cells. □

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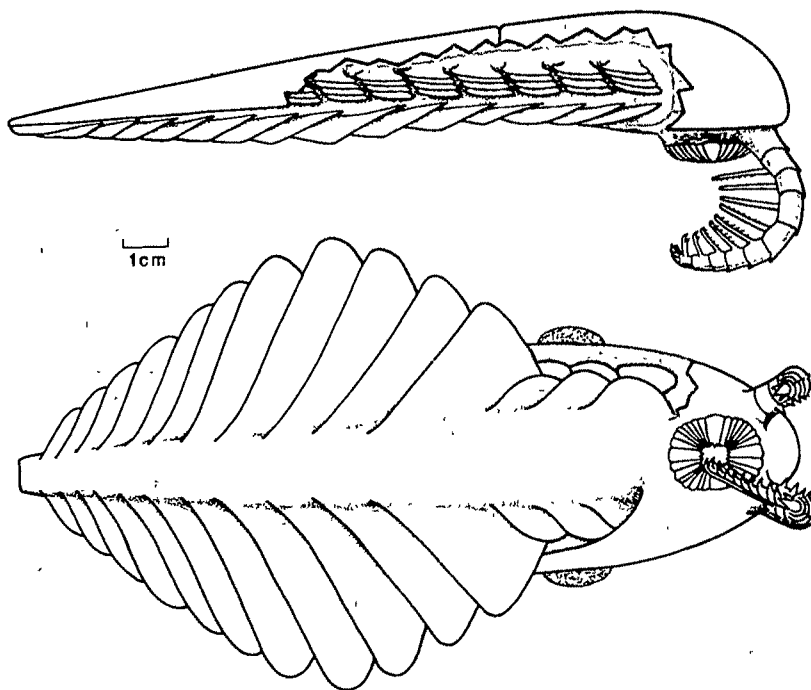
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A Cambrian enigma



Reconstructed lateral and ventral views of *Anomalocaris nathorsti*, appendage partly flexed, lateral lobes in still position. In the lateral view, a portion of external cuticle is cut away to show the arrangements of gills. For the ventral view, part of head region cuticle is cut away to show flaps and gills, and blades are omitted from the right appendage to expose the circling of mouth plates (From *Phil. Trans. R. Soc.* B309, 569, 1985).

How well do we know the fossil record? Recently H.B. Whittington and D.E.G. Briggs (*Phil. Trans. R. Soc.* B309, 569; 1985) have described in detail yet another surprise from that almost inexhaustible palaeontological treasure-house, the Middle Cambrian Burgess Shale of British Columbia (see *Nature News & Views* 302, 570; 1983 and 314, 403; 1985). *Anomalocaris*, the bizarre metazoan shown above has had a chequered scientific history; its isolated jointed appendages have been known for many years from both the Lower and Middle Cambrian of North America. They were generally regarded as the detached limbs of a large arthropod. With the discovery of complete specimens, however, a far more bizarre and enigmatic creature has emerged, with two varieties presently recognized (species or sexual dimorphs?). With a length of probably up to half a metre, it is the largest known Cambrian animal. The body was flanked by flexible lobes that served in propulsion, while gills were housed beneath lateral extensions. A single pair of appendages arose from the underside of the head to flank a remarkable jaw that consisted of a series of plates ending in sharp prongs that encircled the mouth. Although the gut contents are not identifiable, the evidence points to *Anomalocaris* being a predator.

Whittington and Briggs' account is important for our understanding of early metazoan life. Until recently, most palaeontologists believed Cambrian predators rare, exerting a negligible influence in marine communities, although some

years ago G.E. Hutchinson (*Am. Ass. Adv. Sci. Publ.* 67, 85; 1961) suggested that this was an artefact of the fossil record that favours suspension and deposit feeders with robust skeletons (Schopf, T.J.M. *Paleobiology* 4, 261; 1978). *Anomalocaris* is the latest, if most ferocious, addition to the lightly skeletonized and soft-bodied predators that have been found in the Burgess Shale (Conway Morris, S. *Phil. Trans. R. Soc.* B 307, 507; 1985). At some horizons that have yielded *Anomalocaris* there are also trilobites showing evidence of unsuccessful attack, and Whittington and Briggs speculate on how the action of the jaw apparatus can be reconciled with the damage. But some injured trilobites are not associated with *Anomalocaris* and perhaps represent the unwelcome attention of other large predators that so far have eluded discovery (Conway Morris, S. & Jenkins, R.J.F. *Alcheringa* 9; in the press).

The Burgess Shale contains an extraordinary variety of metazoans which, if found alive today, would probably be hailed as new phyla. *Anomalocaris* is no exception; despite the arthropod-like anterior appendages, the rest of the anatomy is irreconcilable with the arthropod bodyplan and the jaw apparatus appears to have no parallel in the Metazoa. It is a reminder of how little we know about the early diversification and ecology of the metazoans.

Simon Conway Morris

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Cell biology

Yeast as the universal cell

from Ira Herskowitz

"In ten years, yeast has changed from *E. coli* with a nucleus to the universal cell." This comment by Lee Hartwell (University of Washington) opened a recent conference of yeast cell biology*, reflecting the two main recurring themes of the meeting. The first was that yeast shares with animal cells many of the molecules and processes that are the objects of intensive study in modern cell biology. It has, for example, ubiquitin, calmodulin, clathrin, endocytosis, peptide growth factors, small nuclear RNAs, retroviral elements and those staples of cell structure and function, actin and tubulin. Thus, such central problems as protein localization, function and synthesis of the mitotic apparatus, and the workings of the cell cycle can all be productively studied in yeast. The second theme was that just about any biochemical, cytological or genetic technique or experimental strategy can be applied in studies with yeast. What distinguishes it from other eukaryotic cells for studying cell biology, however, is its accessibility to the full range of both traditional and modern genetic methods. The power of these techniques to elucidate such fundamental processes as mitosis, endocytosis and the control of the cell cycle was well illustrated at the meeting.

The most powerful of the modern genetic strategies available is to produce mutations in cloned yeast genes *in vitro* and then to replace the wildtype gene with the mutant by recombination *in vivo*. This makes it possible to link biochemical information on a protein *in vitro* with cytological information on structures *in vivo* by studying the behaviour of the mutant. A model analysis of this sort has been carried out by G. Payne (University of California, Berkeley) on clathrin, which is in coated vesicles² and is believed to mediate endocytosis. An antiserum to purified clathrin allowed the gene encoding the heavy chain of the protein (*CHC1*) to be fished out from a library, disabled by mutation *in vitro* and then replaced in yeast. The first result with the *in vitro* clathrin mutant (*chc1*⁻) is that it is, perhaps surprisingly, viable, although the colonies it forms are small. Further studies should help to define the role played by clathrin in yeast. Are the mutants defective in endocytosis, in cell fusion events (such as occur in mating), or in turnover of cell surface material?

The mitotic apparatus

Much work in cell biology is focused on how the mitotic apparatus is assembled and disassembled during the cell cycle and

how it distributes chromosomes to daughter nuclei. An initial goal is to define the proteins that make up the spindle on which the chromosomes become arrayed and then to determine how they function³.

The yeast spindle is a tripartite structure, consisting of microtubules that join at one end to the centromere (the yeast version of the kinetochore of the chromosome) and at the other end to the microtubule organizing centre, the spindle pole body (analogous to the centromere)⁴. The molecular outlines of the yeast mitotic apparatus are now emerging through information from the following sources.

The central element of the structure is the microtubule, composed of tubulin. The story described by D. Botstein (Massachusetts Institute of Technology) for the budding yeast *Saccharomyces cerevisiae* and by M. Yanagida (Tokyo University) for the fission yeast *Schizosaccharomyces pombe* is the same: there are three tubulin (*TUB*) genes, two of them for alpha tubulin (only one of which is essential) and one for beta tubulin. Microtubules are needed for chromosome distribution: inactivating an essential tubulin gene by mutation has the same consequences as adding the popular anti-mitotic agents, nocodazole and benomyl. A suggestion that microtubules might also be involved in bud growth seems not to be borne out, as mutants lacking an essential tubulin gene nonetheless form buds. A crucial question in the mechanism of mitosis is that of the role of the microtubule-associated proteins, which are often speculated to play prominent roles in governing microtubule association and dissociation. They have now been identified in yeast by traditional fractionation and polymerization (F. Solomon, Massachusetts Institute of Technology), and the analysis of mutants produced by gene replacement should quickly establish what part they play.

The most exciting findings so far have been on two genes that affect the spindle pole body (P. Baum, University of Washington). It has long been hoped that some of the numerous *CDC* (cell division cycle) genes⁵ would affect structural components of the segregation apparatus, and it looks as though this might be the case for *CDC31*. The nucleotide sequence of *CDC31* shows that it has homologies to calmodulin and in particular that it contains calcium binding domains. The *CDC31* protein may therefore be a structural component of the spindle pole body with its properties of association controlled by calcium. The second fascinating gene described by Baum seems to control the number of spindle pole bodies. Remarkably, mutants defective in this gene form

extra spindle pole bodies when the *ESPI* protein is inactive — up to eight can be seen by electron microscopy. The *ESPI* gene product thus seems to stop spindle pole body assembly after each round of their duplication at each cell cycle.

Studies of the *KARI* gene indicate that it may also code for a structural component of the spindle pole body and also demonstrate a novel strategy for the use of cloned genes to generate mutant phenotypes. M. Rose (Massachusetts Institute of Technology) observed that overproduction of the *KARI* protein (achieved by placing the *KARI* gene under control by a regulated, highly expressed promoter) has lethal consequences similar to those of inactivation of the *KARI* product. In both cases there is a block in chromosome distribution. This strategy of creating a mutant phenotype by overproduction of a functional product ought to be useful in mammalian cells. It was suggested that a high level of the *KARI* protein might disrupt spindle function by 'tying up' an essential spindle component. The original mutant allele of the *KARI* gene has a distinct phenotype — nuclei fail to fuse after mating between compatible cells. Such defects have been suggested to be due to a defect in the spindle pole body^{6,7}.

Although the mitotic spindle is a symmetrical structure (at least on the cell-biological drawing board), the *ndc1* mutant described by Botstein may reveal ways in which it is not. These mutants display the remarkable aberration that in some cases the daughter cell receives both sets of chromosomes and in other cases, the mother cell receives both sets. (It is perhaps relevant that *cdc31* mutants can exhibit similar behaviour⁸.) This aberration in chromosome distribution suggests that one half of the mitotic spindle is defective in some manner. Perhaps the *NDC1* protein is a connector between the spindle pole body and microtubules. One would have to make the additional interesting speculation that the two spindle pole bodies differ from each other. Localization of the *KARI* and *NDC1* (as well as *CDC31* and *ESPI*) proteins within the spindle by direct cytological observation will be a critical test of these speculations. *ESPI*, *CDC31*, *KARI*, and *NDC1* are notable examples in which genetic analysis has turned up interesting mutants but whose further characterization relies on cytological methods that are newly available in yeast^{9,10}.

The cell cycle

Yeast genetics has traditionally been a major contributor to the elucidation of the control of the cell cycle, defining the genes necessary for the initiation, progression and exit of cells from the growth cycle to various specialized shunts, such as meiosis. The *CDC* genes, which control progression, have recently attracted particular attention because of the possibility that they may have functional homologies to

*The Cetus-UCLA Symposium on Yeast Cell Biology, was

cellular oncogenes of animal cells¹¹. There was therefore considerable interest in a report at the meeting from S. Reed (University of California, Santa Barbara) on *CDC28*, mutations that cause arrest in G₁ without any inhibition of protein synthesis, by contrast with numerous other mutations and with nutritional starvation that result in arrest in G₁ without protein synthesis and are thus less likely to reflect specific controls. The nucleotide sequences of both *CDC28* from *S. cerevisiae*¹² and the functionally related gene *CDC2* of *S. pombe*¹³ show clear homology to protein kinases and Reed reported that the *CDC28* product has protein kinase activity¹⁴. Defining its targets may thus provide insights into key regulators of the cell cycle.

Mating between *S. cerevisiae* cells of opposite mating types, a or α , involves reciprocal arrests of the mating partners in G₁ by oligopeptide pheromones, a -factor and α -factor. Understanding how these agents arrest the cell cycle provides a novel opportunity to study regulation of the cell cycle. Some of the components of this system have now been identified. The working view is that each factor binds to a receptor on the cell surface and then generates an intracellular signal that ultimately leads to arrest. The cells then go on to fuse. The *STE2* gene appears to be a component of the receptor for α -factor (D. Jenness, University of Washington), and the *STE3* gene has been argued to be a component of the receptor to a -factor. The nucleotide sequences of *STE2* (A. Burkholder, University of Washington) and *STE3* (E. Jarvis, University of Oregon) show that both code for several long hydrophobic stretches and hence exhibit hallmarks of membrane proteins. Do *STE2* and *STE3* proteins make up channels of some sort? Only the appropriate receptor components are produced in a and α cells because the *STE2* gene is expressed only in a cells and the *STE3* gene only in α cells, due to the action of regulatory proteins coded by the yeast mating type locus¹⁵. Evidence that response to a -factor and α -factor proceeds by a common intracellular signal was presented by G. McCaffrey (University of Oregon) in experiments using α mutants that produce the receptor normally found on a cells.

Regulators involved in the decision to leave the mitotic cycle and enter the pathway of meiosis and spore formation have been identified. Prior genetic analysis pointed to the *RAN1*¹⁶ product of *S. pombe* and the *RME1*¹⁷ product of *S. cerevisiae* as such regulators. Recent analysis in my laboratory confirms that *RME1* governs entry into meiosis. In particular, the *RME1* product appears to be an inhibitor of meiosis and sporulation: these processes occur only in cells whose *RME1* gene is turned off (which occurs naturally in a/α diploid cells). Determining the manner by which the *RME1* and *RAN1* products act

(as a repressor or a protease?) and identifying their targets, ought to provide insights into the early steps of meiosis.

The stage seems set to learn a great deal from yeast about fundamental cellular processes that have historically been considered within the domain of cell biology. Yeast geneticists no longer flinch at cell

biologist's jargon: clathrin, tubulin, ubiquitin — while cell biologists are learning a new three-letter code: *TUB*, *CDC*, *ESP*. This meeting, the first devoted to yeast cell biology, was well timed to show that contemporary cell biology and yeast research, both areas of great excitement, are joined for a productive future. □

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Ichthyology

Filter-feeding on a grand scale

from Jared M. Diamond

ON 29 November 1984 a second specimen of the megamouth shark *Megachasma pelagios* was captured near Los Angeles, California¹. The original specimen caught off Hawaii in 1976 and possibly the most significant discovery of a marine vertebrate for a decade, constituted not only a new species and genus but also a new family within the shark order Lamniformes^{2,3}. Of particular interest are megamouth's feeding habits, which may be unique among animals and on which the Los Angeles specimen sheds further light.

Among the over 300 specimens of sharks, *Megachasma pelagios* is one of only three that are filter-feeders; the others are macropredators and scavengers. Filter-feeders strain many small food items out of water or mud either by pumping the medium through a stationary filter, as in the case of sessile invertebrates such as bivalve molluscs or polychaete worms, or by pushing the filter through the

medium, as do flamingoes and many fish. The secret of successful filter-feeding lies in filtering large volumes of medium containing high concentrations of food items. Among filter-feeders are the largest mammals and fish — baleen whales, basking sharks, whale sharks and manta rays — for which the problems of filtering enough food are acute. These large filter-feeders are constrained to live at or near the surface of nutrient-rich waters, such as coastal zones and the Antarctic.

Megamouth, which at 4.5 m in length and 750 kg in weight is one of the largest living sharks, is clearly a filter-feeder. The stomach of the Hawaiian specimen contained 1-inch long euphausiid shrimps; shrimps and deep-water jellyfish were found in the Los Angeles specimen. The shark's huge mouth is filled with hundreds of tiny teeth, none over 0.5 inches high. Its highly protrusible jaws can be extended like a hoop-net. Filtering is carried out by a unique type of gill-raker, with a core of cartilage and covered by tiny denticles.

In habitat and anatomy, megamouth is very different from the other two large filter-feeding sharks. The basking shark swims with its mouth open, near the surface of cold nutrient-rich coastal waters. It is a powerful swimmer, with a streamlined body, strongly calcified skeleton, stiff fins, strong skin, and firm muscle and connective tissue. Its filtering apparatus consists of vast gill cavities and streamlined gill-rakers, designed to support a high flow rate. The whale shark is also a strong swimmer that often feeds near the surface and can suck in and filter out water even when stationary.

By contrast, megamouth lives in deep water with lower plankton concentrations



Megamouth shark *Megachasma pelagios* captured off Los Angeles in November 1984 (Photograph courtesy of L. J. V. Compagno).

than surface waters; one specimen was taken at 125 feet or less, the other at about 540 feet. The soft loose skin, flabby muscles and connective tissue, soft cartilaginous skeleton with little calcification, and non-streamlined form suggest it is a slow, weak swimmer, and explains why megamouth is the only shark known to be attacked by the 'cookie-cutter shark', *Isistius brasiliensis*, so-called because of the neat pieces it bites out of large fish and porpoises⁴. Its inefficient filtering mechanism, with a small gill cavity, restricted internal gill apertures and non-streamlined gillrakers, is ill-suited for a high flow rate. Instead, it has a huge tongue that almost fills the mouth when closed, presumably to expel water across the filter with jaws shut.

How could such a large but weak animal, with a low-flow filter and living well below the rich plankton zone, filter enough plankton to support itself? A clue comes from the bright silvery lining of the mouth, suggesting that it is bioluminescent. The stomach contents include species that live in the deep scattering layer and that are known to be bioluminescent. Thus, megamouth may feed by holding its mouth open in the dark, attracting plankton like moths to a light trap. Some abyssal anglerfish have bioluminescent organs to attract their fish prey, but I am unaware

of a filter-feeder other than megamouth that uses light to attract plankton.

The closest parallel to megamouth's suggested feeding strategy may be found in frogmouths (family Podargidae), which are large nocturnal birds of New Guinea and Australia. Although frogmouths are well known to pounce on large insects and small vertebrates, this foraging technique fails to explain why they have a huge mouth. However, *Podargus papuensis* of New Guinea spends much time perched with its mouth wide open, perhaps using an odour to attract aerial plankton on to the sticky paste on its palate. If evidence is forthcoming to support the suggestion that megamouth sharks and frogmouths attract plankton into their open mouths by light and chemicals, respectively, the infrequency with which such behaviour has evolved will be puzzling. Why do almost all filter-feeders go to great efforts to pump water through their filters or to push their filters through water, instead of attracting their prey into their mouths? □

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Atherosclerosis

Scavenger cell receptor shared

from Michael S. Brown and Joseph L. Goldstein

WHEN a blood vessel is damaged, scavenger cells (macrophages) migrate into the wound where they ingest and degrade the debris that has escaped from the bloodstream. But problems with the normal process can arise within the walls of arteries, because the macrophages ingest, among other things, the cholesterol-carrying low density lipoproteins (LDL) of plasma that have penetrated into the artery wall. The insoluble cholesterol of LDL cannot rapidly be excreted and accumulates in the macrophages as lipid droplets, converting the macrophages into 'foam cells' that contribute substantially to the mass of atherosclerotic plaques¹. Our understanding of atherosclerosis would be advanced if we knew how macrophages identify abnormal lipoproteins and other debris for ingestion in preference to normal extracellular components. Answers are emerging from studies of a cell surface receptor on macrophages that binds chemically altered proteins and on page 746 of this issue, Phillips and colleagues show that platelets release a substance that affects this receptor².

Surprisingly, normal macrophages express very few LDL receptors of the classic type that is found on fibroblasts and liver cells³. They therefore ingest native

LDL sparingly. When LDL is, however, modified chemically by acetylation, it loses the ability to bind to the classic LDL receptor, but is now recognized by another high-affinity receptor on macrophages³. The receptor-mediated uptake of acetyl-LDL leads to massive deposition of cholesterol within the macrophage^{1,3}. The receptor that mediates this uptake has been called the acetyl-LDL receptor or the scavenger cell receptor¹. It also recognizes LDL and other proteins which have been treated with agents that alter lysine residues, including succinic anhydride, maleic anhydride and malondialdehyde^{1,4}. As with acetylation, these agents increase the net negative charge of the protein. Other negatively charged compounds, such as sulphated glycosaminoglycans also bind to it. Gotto and coworkers have recently solubilized and partially purified the acetyl-LDL receptor from the mouse macrophage cell line P338D₁. By ligand blotting, they have shown it to be a protein of approximate relative molecular mass (M_r) 260,000 (ref. 5), clearly different from the 160,000 M_r LDL receptor.

Although the acetyl-LDL receptor lacks absolute chemical specificity, it is cell specific. It seems to be present only on

macrophages¹ and endothelial cells^{6,7}. Endothelial cells are not usually considered to be scavenger cells because they show little phagocytosis of particulate matter⁸, but the discovery that they have acetyl-LDL receptors resurrects the possibility that they share some scavenger functions with macrophages. In the body the acetyl-LDL receptor is particularly abundant on the endothelial cells that line the hepatic sinusoids. When fluorescent or radioactive acetyl-LDL or acetoacetyl-LDL is administered to animals intravenously it rapidly accumulates in the hepatic sinusoidal endothelium and to a lesser extent in hepatic Kupffer cells⁹⁻¹¹.

There remains a crucial question — the identification of the natural ligand for the acetyl-LDL receptor. It seems unlikely that lysine residues would be acetylated in extracellular fluid *in vivo*. On the other hand, Steinberg *et al.* have observed that LDL can be converted by chemical oxidation into a negatively charged form that binds to the acetyl-LDL receptor¹². When LDL accumulates in extravascular spaces, it might undergo oxidation, thereby increasing its net negative charge, as well as permitting uptake by the acetyl-LDL receptor of macrophages. Such a mechanism might explain the occurrence of cholesterol-loaded foam cells in atherosclerotic plaques, and also the accumulation of cholesterol in macrophages throughout the bodies of patients with homozygous familial hypercholesterolaemia. Indirect support for this hypothesis comes from the finding that LDL isolated from inflammatory tissues of rabbits has an enhanced negative charge¹³.

Phillips *et al.*² add a new twist to the story. They show that activated platelets release a substance of $M_r > 100,000$ that blocks the uptake and degradation of acetyl-LDL by macrophages, apparently as a result of competitive binding. This substance is likely to be one or more of the negatively charged proteoglycans that are stored in platelet granules and released after thrombin treatment¹⁴. Secretion of this substance might limit the ability of macrophages to clear lipoproteins from extracellular deposits. In addition, this

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platelet-derived substance might serve as a chemotactic agent to attract blood monocytes to sites of tissue injury and to activate macrophages at these sites by interaction with the acetyl-LDL receptor.

The emerging story suggests that the acetyl-LDL receptor may mediate scavenger functions that are shared by sinusoidal endothelial cells and macrophages. At present, however, there is no direct evidence to link this receptor with cholesterol accumulation in atherosclerotic plaques. Possibly the physiological

ligand for this receptor is not a lipoprotein at all but some other negatively charged macromolecule, such as the one released from platelet granules⁵. The receptor is now being purified⁵, so it should soon be possible to prepare antibodies against it, thus helping to remove some of the mystery surrounding its function. □

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Oncogenic intelligence

Another link in the chain

from Peter Newmark

IT CAME as a complete surprise when one chain of platelet derived growth factor was revealed to be very similar to that of the product of the *sis* oncogene and, again, when the epidermal growth factor receptor sequence was found to contain the sequence of the *erb-B* oncogene product. But, if the sequence of the *fms* oncogene product turns out to be identical to part of that of the receptor for the growth factor CSF-1, it will be no surprise, following the publication by C.J. Sherr *et al.* of highly suggestive evidence of a close relationship between the two proteins in *Cell* 49, 656; 1985. This forging of a third link between the genes for growth factors or their receptors and oncogenes will add impetus to the quest to discover whether the mutation of the factor or receptor genes is a contributory cause of human cancer.

Of the two proteins compared by Sherr *et al.*, more is known of *fms*. Like most oncogene products, it was discovered in an animal tumour virus, in this case the McDonough strain of feline sarcoma virus. A good deal has been learnt by Sherr's group about the properties of both the viral protein, which is encoded by the *fms* oncogene (*v-fms*), and the protein that is encoded by the *fms* proto-oncogene (*c-fms*) — a gene of mammalian cells and the progenitor of *v-fms*. Less is known of the functions of either protein. The oncogene product obviously transforms normal cells into tumour cells. It also comes into the category of oncogene proteins that are located on the cell surface and are associated with an enigmatic ability to catalyse the phosphorylation of tyrosine residues in proteins; a similar enzymatic activity is associated with the product of the proto-oncogene. Particularly by analogy with *erb-B*, these facts have suggested that *c-fms* encodes a growth factor receptor and that *v-fms* encodes part thereof but with a truncation or other alteration that subverts the normal function of the receptor.

But which receptor is it? The probable answer has come from studies by E R

(colony stimulating factor-1), which is a protein that stimulates the production of macrophages from their precursor cells. The factor binds specifically to cells of the mononuclear phagocytic series, including macrophages, and seems to have some characteristics that are reminiscent of the *c-fms* product.

To cement the relationship between their two proteins, Sherr's and Stanley's groups have now obtained two sets of data, which they jointly report in *Cell*. The first establishes the presence of a *c-fms*

product with an associated tyrosine kinase on the membrane of macrophages, as it should be if it is the CSF-1 receptor. The second demonstrates that antibodies to the *v-fms* product recognize a macrophage membrane protein that binds CSF-1 and has other properties of CSF-1 receptor.

Now we need sequence data. The sequences of *v-fms* and, by deduction, its product are known, but those of *c-fms* and the CSF-1 receptor are not. Only when they become available will it be possible to be certain that the *c-fms* gene encodes the CSF-1 receptor and to see what alterations have accompanied the conversion of *c-fms* into *v-fms*. As Sherr *et al.* say, there are two possibilities. One is that, parallel to the *v-erb-B* gene, *v-fms* may lack the sequence that encodes the CSF-1 binding domain of the receptor and could encode a tyrosine phosphorylating activity that is constitutively active rather than CSF-1 stimulated. But since the *v-fms* product does not seem to be seriously truncated, Sherr *et al.* make the alternative suggestion that the *v-fms* product is a competent receptor and that its delivery by tumour virus to cells that do not normally contain the receptor, particularly fibroblasts, provides them with an abnormal ability to respond to CSF-1, which fibroblasts themselves synthesize. □

Peter Newmark is Deputy Editor of Nature

Particle physics

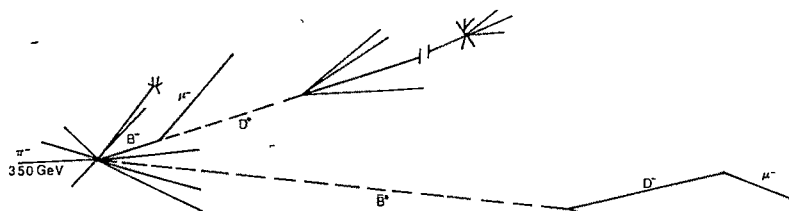
Naked beauty seen at last

from David J. Miller

CLEAR tracks of 'beauty' particles have at last been seen. The existence of the beauty or bottom quark has been accepted ever since the discovery of the upsilon particles in 1977, but the upsilons are the 'hidden beauty' bound states of a beauty quark with a beauty anti-quark. There should also be 'naked beauty' states in which the beauty quark binds with one of the lighter 'up', 'down' or 'strange' anti-quarks, forming B mesons. There has been evidence for some time for the production of B mesons, but only from electron-positron colliding beam machines in which all of the interesting processes happen inside the vacuum pipe. Heroic attempts have been made to deduce the lifetime of the B from these data but the results are not yet completely convincing.

The figure shows the convincing example of the production and decay of a pair of

beauty particles found in a nuclear emulsion stack that had been exposed to a 350 GeV p-minus beam at the CERN super proton-synchrotron by the WA75 collaboration (Brussels, Dublin, CERN, University College and Birkbeck College, London, Torino, Rome, Bari and Nagoya with associated Japanese laboratories). All five vertices are well resolved. The p-minus meson comes in from the left on the sketch and strikes a nucleus in the emulsion. Among the tracks coming from that primary interaction vertex, the B⁻ travels about half a millimetre in the emulsion before it decays to a muon (μ⁻). Just over half a millimetre from the B⁻ decay-vertex there is a 'four-prong' vertex that is taken to be the decay point of a neutral charmed particle, a D⁰ meson. Another neutral decay is seen some millimetres from the primary vertex. This is taken to



be the decay point of an anti- B^0 meson ($\bar{B}^0$) that produces a negative charmed particle, probably a D^- , which itself decays after some millimetres, giving rise to another muon.

The process illustrated in the figure seems very simple, yet scanners using high-powered microscopes took months to put the event together, even after the primary vertex had been located. The 'needles' to be searched for are tracks made up of grains less than a micrometre across. The experiment used 80 litres of Fuji and Ilford emulsion — a 'haystack' of 8×10^7 cubic millimetres. The problem was made manageable both by surrounding the emulsion with electronic detectors which identified the candidate events that were likely to contain beauty particles and by pointing back the tracks from such events to define a search-volume of about a cubic millimetre for each candidate. To avoid overcrowding the emulsion, with lots of beam tracks in one place, it was moved periodically on a precision stage.

Many of the tracks seen in the emulsion correspond to those reconstructed from the high-resolution silicon-strip detectors that were used to predict the search volume. The muon tracks are especially important in identifying beauty candidates and for this event both of them were seen by the silicon detectors and in a downstream muon spectrometer where their momenta were measured after they had passed through a tungsten and steel wall that absorbed the other (strongly interacting) particles. The transverse momentum of the muon from the B^- was particularly large, consistent only with

beauty decay, too big to have come from the decay of a lighter charm particle. The presence of this muon, recognized by fast electronics 'on-line' within a few microseconds of the interaction taking place, gave the primary 'event trigger' that allowed all available information from the detectors to be written on magnetic tape. Analysis of the data off-line picked up the second muon from the D^- decay, which made the event a prime candidate to be notified to the emulsion scanners.

Is one event so important? Even with two beauty tracks it cannot properly measure the beauty lifetime. But the decay times estimated from it are significantly shorter than those from the electron-positron machines, a few times 10^{-13} seconds, as opposed to more than 10^{-12} seconds. The lifetime of beauty is one of the constraints on part of our standard model of the electroweak interaction. Theorists were becoming embarrassed by long beauty lifetimes. The parameters of the model (the elements of the Kobayashi-Maskawa matrix) were becoming strained beyond reasonable limits. The WA75 event suggests that, once again, experimental results may be reinforcing the simplest possible model for weak processes. The group expect eventually to find enough needles in their haystack to measure the lifetime properly. For now, it is good to know for sure that beauty particles exist. □

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Ocean Drilling Program

Looking down an old hole

from the Leg 102 shipboard scientific party*

ONE of the problems of drilling is that it is virtually impossible to extract a complete core from a drill-hole and one can never be certain just what is missing. Fortunately there are a myriad of techniques available to make *in situ* geophysical measurements of the rocks exposed in the sides of a hole. Because such measurements had been so successful in characterizing young (6.2 Myr) oceanic crust at Site 504 in the Pacific, it was decided that Leg 102 should re-investigate Hole 418A on the southern edge of the Bermuda Rise (25° 02'N; 68° 04'W), from which an incomplete core was taken eight years ago as part of the Deep Sea Drilling Project. The geophysical data obtained from these 110-Myr-old oceanic basalts show some interesting differences compared with the young Pacific crust.

Undeterred by the prospect of finding a hole only 25 cm across in water over 5 km deep, the new Ocean Drilling Program's drillship *JOIDES Resolution* arrived

at Site 418 on 21 March, 1985. For the next 2½ weeks borehole geophysical measurements were made to depths of 800 m below the sea floor in the upper 500 m of basalt. The results obtained can be interpreted in terms of ageing of the basaltic crust.

Acoustic velocity and waveform logs showed relatively high compressional and shear wave velocities (4.5–6.0 and 2.1–3.4 kms^{-1} respectively) throughout the upper 500 m of the oceanic crust (layer 2), with slow values occurring in the predominant weathered pillow basalt and breccia units and fast values in the massive basalts, which would be expected to be less prone to alteration. A similar difference was shown by induction and focused electrical resistivity logs, with resistivities of 40–200 ohm-m through the pillow basalts and breccias and values greater than 1000 ohm-m in the massive units. The upper 500 m below sea floor were distinguished by unusually high gamma ray activity

and relatively high formation porosities, as determined by density and neutron porosity logs. The base of this section was marked by a sharp magnetic polarity reversal, followed by a notable low on the magnetic susceptibility log from 500 to 600 m below sea floor, then a gradual increase to the bottom of the log. Unusually for holes drilled in Layer 2, caliper logs demonstrated that the hole was 'to gauge' throughout. But perhaps most excitingly, temperature measurements indicated conductive, rather than convective, heat flow in the old basalt. This suggests a less active hydrothermal regime, a view supported by the water chemistry, which showed a difference between water within the borehole and that in the surrounding rocks, the borehole water being enriched in Ca^{2+} and depleted in Mg^{2+} and K^+ .

Our preliminary interpretation of the borehole geophysical and geochemical data is that the old crust at this site has been sealed by alteration products (clays and carbonates) within the pillow basalt units, and between layers of pillow and massive basalt, restricting the movement of pore water and causing geophysical Layer 2A now to be absent. This is in contrast to the interpretation of similar studies at Hole 504B in young crust in the Pacific. At that site, Layer 2A is present, the upper basalt is relatively porous and permeable, and observed underpressures and flow of seawater into the upper permeable zone suggest weak hydrothermal convection within the pillow lavas.

A problem facing all investigations using drill-holes is to extrapolate from the downhole log to the regional geology. Large-scale seismic experiments may be used to estimate large-scale fracturing and porosity and to determine the velocity structure, anisotropy and heterogeneity of the crust. Such seismic work may also indicate the presence of deep reflectors, for example, the boundary between extrusive and intrusive basalts within the oceanic crust. To meet these objectives, an oblique ship-to-ship seismic experiment was conducted at Site 418 using the RV *Fred H. Moore* as the shooting ship and the *JOIDES Resolution* as the receiving vessel. Shot points were located along radii and concentric circles centred on the borehole. A close-spaced seismic reflection survey was made to obtain basement topographical corrections. Seismic anisotropy and lateral heterogeneity were clearly established. Because of the techniques and equipment used, the data are of high quality, making it possible to extract evidence of deep reflectors, either within the crust, if they exist, or at the Moho. □

*Co-chief scientists: M.H. Salisbury (Scripps Institution of Oceanography) and G.H. Scott (U.S. Geological Survey). Also: C. Auvroux (ODP, Texas A&M Univ.), K. Becker (Scripps), W. Bosum (Federal Institute for Geosciences and Natural Resources, Hannover, F.R.G.), C. Broglia (Lamont-Doherty Geological Observatory), R. Carlson (Texas A&M Univ.), A. Fisher (Univ. of Miami), J. Gieskes (Scripps), M. Holmes (Florida State University), H. Hoskins (Woods Hole Oceanographic Institution), J. Legrand (IFREMER, Brest, France), D. Moos (Lamont-Doherty), D. Rio (Istituto di Geologia, Parma, Italy), D. Seckman (Woods Hole), D. Wilkens (MIT).

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What was all the fuss about?

Richard Dawkins

Time Frames: The Rethinking of Darwinian Evolution and the Theory of Punctuated Equilibria.

By Niles Eldredge.

Simon & Schuster: 1985. Pp.240. \$16.95. To be published in Britain by Heinemann in January 1986.

THE alert reader of scientific magazines can hardly fail to be aware of a widespread lay rumour of something rotten in the state of Darwinism. We have seen a spate of more or less silly books by fundamentalist crackpots, cosmologists, biochemists and BBC producers, all banging the same drum. Two quotations from reviews give the general flavour: "... a highly readable non-technical account of just why Darwinism has come to be seen as a totally inadequate account of how life came to evolve on this planet"; "An original challenge to an increasingly beleaguered orthodoxy". There is nothing original in the objections deployed by these books, just the old favourites ("The eye is too complicated to have evolved by blind 'chance'", and the rest) that Darwin himself raised — and demolished. These arguments have been repeatedly rediscovered, and the answers to them repeatedly ignored or misunderstood, during each of the decades since Darwin first discussed them. So why have gleeful rumours of a "beleaguered orthodoxy" spread particularly in the latest decade? Has there been some fresh impetus, some source of new encouragement for the old fundamentalists and fellow travellers?

Like any vigorously flourishing field of science, Darwinism has always had its in-house disputes. If these had been noticed by a wider public, it is likely that they would have been seized upon as evidence that the Darwinian "orthodoxy" was crumbling, so ready is the motivation to find such evidence. The effect is heightened if any chink of encouragement is offered by professionals on the inside of the business. I regret to say that more than a chink was offered by champions of "punctuated equilibria". The debate about this interesting little theory is a technical, parochial affair if ever there was one. It lies firmly *within* the neo-Darwinian synthesis. It is no more revolutionary than many another argument that has enriched the synthetic theory, for instance that over the "gene as the unit of selection". Yet punctuationism is widely thought to be revolutionary and antithetical to neo-Darwinism, for the simple reason that its chief advocates have said that it is; said so, moreover, in loud and eloquent voices, making frequent and skilful use of the mass media. The theory, in short, stands out from other glosses on the neo-Darwinian synthesis in one re-

spect only: it has enjoyed brilliant public relations and stage management.

Dr Eldredge is one of the two originators of the theory of punctuated equilibria. He is neither a cosmologist nor a biochemist, but a genuine expert on evolution. He has to be taken seriously, and he offers us here a pleasantly written account of the punctuation theory. The book is partisan, and none the worse for that. It is engagingly personal: one has a glimpse of what it is like to be a good palaeontologist and to love trilobites. It lapses into skipworthiness only when Eldredge indulges his predilection for woolly analogies with the social sciences, and his curious enthusiasm for someone called Frederick Teggart.

There is a useful reprinting of the original paper by Eldredge and Gould which launched the theory of punctuated equilibria in 1972. Re-reading this paper, and reading the new book, I was struck by the comparative modesty of both. The more self-aggrandizing hype seems to have been a phenomenon of the intervening years, climaxing, perhaps, in 1980 when Stephen Gould pronounced the

synthetic theory "effectively dead", and when a science journalist, sycophantic enough to deserve the title Gould's Poole, hailed "An historic conference in Chicago" which "challenges the four-decade long dominance of the Modern Synthesis". If the book signals the end of that grandiloquent era it is very welcome, for the misunderstood rhetoric of middle-period punctuationism gave abundant aid and comfort to creationists and other enemies of scientific truth, and may well have been largely responsible for the ill-informed rumour that I mentioned at the outset. It is only fair to add that both Eldredge and Gould have been latter-day bulldogs in the fight against American fundamentalism.

Darwin's own bulldog, Huxley, as Eldredge reminds us yet again, warned him against his insistent gradualism, but Darwin had good reason. His theory was largely aimed at replacing creationism as an explanation of how living complexity could arise out of simplicity. Complexity cannot spring up in a single stroke of chance: that would be like hitting upon the combination number that opens a bank vault. But a whole series of tiny chance steps, if non-randomly selected, can build up almost limitless complexity of adaptation. It is as though the vault's door were to open another chink every time the number on the dials moved a little closer to the winning number. Gradualness is of the essence. In the context of the fight against creationism, gradualism is more or less synonymous with evolution itself. If you throw out gradualness you throw out the very thing that makes evolution more



The Chinese way of bookselling — detail of a bookshop of Northern Sung appearing in a long scroll painting, Spring Festival on the River (early twelfth century AD). The illustration is taken from Part I, Paper and Printing, by Tsién Tsuen-Hsün, of Vol. 5 of Joseph Needham's Science and Civilisation in China. Publisher is Cambridge University Press, price £45, \$89.50.

plausible than creation. Creation is a special case of saltation — the *saltus* is the large jump from nothing to fully formed modern life. When you think of what Darwin was fighting against, is it any wonder that he continually returned to the theme of slow, gradual, step-by-step change?

Punctuationists are in favour of a form of jumpy evolution, but it is leagues away from the saltationism that Darwin was fighting. The punctuationists' "real gaps" in the fossil record are gaps only if you confine your digging to one place. They are all too easily confused with the sort of gaps that only a divine architect could bridge. In Darwin's sense of the term, Eldredge and his colleagues must be gradualists — they would have to be creationists if they weren't. But they believe that the episodes of step-by-step, gradual evolutionary change are sporadic, separated in space and therefore hard to find in the fossils, and separated in time by long periods of stagnation. All that the punctuationists have really done is to inject periods of stagnation into an otherwise traditional Darwinian picture of gradual evolution with allopatric speciation. Whether they are right to do so is an empirical matter that palaeontologists continue to debate. The upshot of this factual debate, whichever way it goes in particular cases, will upset nobody, and certainly not "the four-decade long dominance of the Modern Synthesis". Darwin himself would have been equally happy either way, because evolutionary change is still gradual *during the times that it is actually happening*. The punctuationists deluded themselves that they were saying something revolutionary. And it was all due to a simple verbal misunderstanding: a confusion of two senses of the word "gradual".

There is a second controversy which does, in a sense, move outside the neo-Darwinian synthesis, narrowly interpreted. This is about whether a form of natural selection operates at the level of entire lineages, as well as at the level of individual reproduction stressed by Darwin and neo-Darwinism. Eldredge is particularly involved with this now and he makes some worthwhile points, especially about the "effect hypothesis", and about the unitary status of the species as a player in the evolutionary game. But he agrees that species-level selection can't explain the evolution of adaptations: eyes, ears, knee joints, spider webs, behaviour patterns, everything, in short, that many of us want a theory of evolution to explain. Species selection may happen, but it doesn't seem to *do* anything much. After reading this book, one is left with a feeling of "What was all the fuss about?", and a suspicion that Eldredge may be left with it too. □

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Unseen horizons: the future of astronomy

George Efstathiou

The Invisible Universe: Probing the Frontiers of Astrophysics. By George B. Field and Eric J. Chaisson. *Birkhäuser*: 1985. Pp.195. SwFr. 58, \$19.95.

THE unveiling of the invisible Universe represents the most significant development in astronomy of the twentieth century. Since Galileo's time, astronomers have observed the sky with optical telescopes using visible light. Professors Field and Chaisson focus on the rest of the electromagnetic spectrum — the invisible radiation which holds the clue to many of the most spectacular phenomena in our Universe.

Their account is based loosely on a major report, *Astronomy and Astrophysics for the 1980s*, which was compiled for the United States Federal Government by a committee chaired by Professor Field. In the book Field and Chaisson provide a popular account of the advances we can expect from the next generation of satellites and telescopes. The choice of subject matter is wide-ranging — from Solar Sys-

tem astronomy to high-energy particle physics — and with the strikingly beautiful colour pictures cannot fail to excite the reader's imagination. The discussion is lucid, informed and supported by a useful glossary, although in places (for example the section on interstellar molecules) it does become quite technical and probably beyond the understanding of most lay readers. Throughout the book, the glittering array of new facilities provides the central theme but, curiously, the authors do not distinguish between those that are approved, such as the Space Telescope, and those that may never be built.

In some places, the discussion is too brief to be very helpful. For example, the Field committee concluded that an explanation for the vast amounts of energy produced by quasars is one of the most important of the current problems in astronomy, and so it was disappointing to find that only a few pages are devoted to the puzzle. Nevertheless, I enjoyed this book. Anybody who wants an authoritative account of future directions in astronomy would do well to read it, and it will be especially useful to undergraduates who are thinking of doing research. □

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Coming devices

C.R. Pidgeon

Free-Electron Lasers. By Thomas C. Marshall. *Macmillan, New York/Collier Macmillan, London*: 1985. Pp.191. \$24.95, £29.95.

Two years ago one could say that although many experiments had demonstrated the free-electron laser (FEL) as an amplifier of laser light, the gain threshold for actual laser oscillation in a mirrored optical cavity had only twice been surpassed. A renewed burst of activity since then has resulted in demonstrations of FEL oscillation in many countries, and at wavelengths all the way from the millimeter to the visible region of the spectrum. At the most recent meeting of the regular series of FEL conferences (Rome, 1984) it was predicted that in the near future ultraviolet, vacuum ultraviolet and soft X-radiation will be generated by FEL devices. As Thomas C. Marshall states in the present work, the FEL will be increasingly leaving the designer's laboratory and becoming available for research.

In a FEL amplifier one observes or infers the amplification of an external laser beam as it interacts with an undulating electron beam passing through the periodic magnetostatic structure of a "wiggler". By contrast, an FEL oscillator

produces a laser beam without the need for an external laser. The spontaneous radiation generated by undulating electron bunches in the wiggler is reflected back and forth between a pair of end mirrors, generating enough stimulated emission in successive electron bunches to produce and sustain a laser beam.

Marshall has provided a worthwhile treatment of the operation and characteristics of the FEL, with a useful historical and conceptual review, and bibliography. Most aspects of the subject are covered, including both single-particle (or Compton) systems and collective-particle (or Raman) systems. The author has attempted — reasonably successfully, I think — to write for the larger scientific community something between "news" and a professional review paper. The book will also be useful to the FEL community itself as a less-specialized source of information in what has by now become quite a broad and fast-moving field, though the most detailed and up-to-date information must of course still be obtained from the proceedings of the yearly workshops held on FEL physics and devices. The only slight quibble I have, perhaps inevitable for a book of this length, is that in several places quite complex results are merely quoted, without derivation or even comment from other sources. □

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Thinking about the ways of science

John Ziman

Popper Selections.* Edited by David Miller. Princeton University Press: 1985. Pp.479. Hbk \$32.50; pbk \$9.95.

ANY natural scientist interested in the philosophy of science will be acquainted with the work of Karl Popper. His identification of "falsifiability" as the hallmark of a scientific theory is now so widely accepted that scientists even use it as a weapon in purely scientific controversies: "I know my theory is conjectural, but at least it could be refuted by the following experiment, whereas yours is so broad and vague that it could not be disproved" etc., etc.

Many scientists attracted to Popper's ideas will have read their way with profit and pleasure through his half-dozen or so major books, and come to appreciate the breadth and depth of his thought on the meaning and social significance of the scientific approach to the world — otherwise one would have had to be content with a version of these ideas at second hand, in one of the books that have been written about them by other philosophers. But Popper is not one of those gurus whose jewels can only be appreciated by cleaning away a mass of obscuring verbiage. Not all his writing is perfectly clear and transparent at the first reading — he is dealing, after all, with very difficult questions — but there are many passages that sum up his arguments with extreme clarity and simplicity. David Miller's selection of some of these passages is thus an invaluable introduction to the work of one of the leading thinkers of our times.

It is not difficult to understand why working scientists are drawn to Popper's philosophy. His characterization of the work of research as the evolution of a body of knowledge through a process of trial and error resonates with their own experience, both as would-be discoverers and as critics of discovery claims. This overall account of the scientific process has, of course, been attacked in detail by numerous other philosophers, but it still stands firm. Indeed, the most striking feature of Popper's thinking and writing is that it is so *robust*. He fastens on a few very solid principles, and stands firmly on them throughout — the corrigibility of all scientific knowledge, the logical force of a disconfirmed prediction, the vapidness of philosophical idealism and, in the social sciences, the absurdity of the assumption that people always behave rationally. Knock-me-down arguments are not welcomed in professional academic life, which thrives on subtlety and equivoca-

tion, but I really wonder how the modern exponents of the sociology of knowledge would answer Popper's devastation of their position, originally published 40 years ago and still entirely valid.

Of course there are situations where reliance on a few apparently sound general principles can be misleading. Popper was once led into asserting that the principle of biological evolution was not a "scientific" hypothesis because it seemed essentially tautological: biologists will be pleased to read here a passage written in 1977 where he gracefully acknowledges his error on this point. I suspect that his remarks on "The Mind-Body Problem" and "The Self" will not stand for long, as natural,



Man of principles — Karl Popper, a portrait from around 1950.

and social and behavioural scientists collaborate more closely in their accounts of consciousness and cognition. It is also surprising that the very clear conception of the "public" character of the scientific process, through the intersubjectivity of observation and the mutuality of creative criticism, which he gave in a page or two in 1945, scarcely figures in his later work. Again, his notion of "world 3" — that is, the domain of "the objective contents of thought", such as scientific theories and works of art — could have been made much more convincing and fruitful if it had been firmly related to the function of symbolic communication in human societies.

But in the end one comes back to Popper for his incisive commentary on the *strategies* of scientific and political action. He does not provide us with *rules* on which to act — indeed, his first rule would be that there are no such rules — but his critique of scientism and historicism is founded upon *precepts* which every scientist, every citizen, would ignore at their peril. □

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Speechless in Java

John C. Marshall

Ancestral Voices: Language and the Evolution of Human Consciousness. By Curtis G. Smith. Prentice-Hall: 1985. Pp.178. Hbk \$14.95, £17.50; pbk \$8.95, £9.60.

THE "ancestral voices" that Kubla Khan heard in Xanadu were "prophesying war"; the voices that appear in Curtis Smith's title are those of our purported evolutionary precursors who first developed the capacity for symbolic communication. But the parallels are somewhat too close for comfort. The archaeological record, Smith conjectures, suggests that linguistically-skilled Cro-Magnon "with his superior culture and technology" exterminated speechless Neanderthal man, "his closest rival for the riches of Europe".

Like so many eminent scholars before him, Curtis Smith, a professor of biological sciences at Mount Holyoke College, Massachusetts, has failed to resist the temptation to speculate on the evolution of language and consciousness. Professor Smith's mixture is fairly conventional: fossil remains, flint tools and progressive enlargement of the cerebrum, combined with attempts to teach chimpanzees American Sign Language and to communicate with hypothetical intelligences on other planets. The problem, however, is that whilst we have undoubtedly learnt much about the structure of the brain and much about the structure of human language we still have few (or no) coherent ideas about how to relate the two. That localized damage to different parts of the brain can result in differential loss of aspects of linguistic capacity and skill places constraints upon speculation but leaves the central issue untouched. Professor Smith has no more insight into that issue — how the brains of human beings differ from those of other higher primates such that only human brains can support a syntactically-structured language — than do the rest of us.

This leaves Smith to make some none-too-accurate general remarks about the functional organization of the brain. Thus he asserts that the brain resembles "an enormous hologram" in that "all of the neural symbols used for language, visual memories, music, feelings, are stored 'everywhere' ". It may turn out that this claim is true, but it is grossly misleading to present the holographic hypothesis as already established fact; and it is even worse to do so without giving any indication of how such distributed information-storage could be compatible with the specificity of deficit that is so often observed after focal damage. Smith's convictions are rarely backed by evidence. He simply states, for example, that "amnesias are defects in the retrieval system, not actual

*In Britain published by Fontana as *A Pocket Popper*, pbk £4.95.

loss of the memories themselves" without indicating that such matters are still under active debate. He is likewise convinced that the storage and recognition of faces is a holographic process, but does not bother to mention that in (monkey) cortex there do appear to be single cells tuned to particular familiar faces. His interpretation of commissurotomy ("split-brain") patients also prejudices the outcome of current discussion. *Contra* Smith's confident assertions, there are reputable scientists who do believe that we have two brains (hemispheres) and that (in the vast majority of people) language capacity and skill is limited to one of them.

Ancestral Voices is clearly intended as a popular, "lay" book on a topic that very naturally fascinates many people well beyond the confines of the neuroscience community. All the more important, then, that a fair picture should be painted

of the *limits* of current knowledge, and of the disagreements between those who work in the area. Professor Smith's efforts do not meet these requirements. Whitney's trenchant summary (1870) of theories of the origin of language comes to mind all too often:

No theme in linguistic science is more often and more voluminously treated than this, and by scholars of every grade and tendency; nor any, it may be added, with less profitable result in proportion to the labor expended.

If, despite Whitney's pessimism, the urge to read a book on the topic remains, I would recommend that one searched for a copy of George John Romanes' Victorian masterpiece, *Mental Evolution in Man* (1888). □

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Temporal questions

James A. Secord

The Dark Abyss of Time: The History of the Earth and the History of Nations from Hooke to Vico. By Paolo Rossi. Translated by Lydia G. Cochrane. University of Chicago Press: 1985. Pp.338. £32.25, \$35.

ANYONE familiar with the development of science in the past few decades will recognize that disciplinary boundaries are far from static. Sciences move closer to and further away from one another; they break up, disappear or regroup into new subjects and specialities. Move back 300 years, and the changes involve not only science but the whole realm of human knowledge. What, for example, do fossil ammonites have in common with Chinese writing and the Egyptian pyramids? To modern ears the question seems absurd, for these subjects now belong in very distinctive scientific specialities, and any relation between them would seem a matter of chance. But in the seventeenth century their affinity was no riddle, but a feature of intellectual life that was taken for granted. Fossils, ancient languages and relics of lost civilizations all had a place in a unified study of the past: they were "signs of time". Hence the original Italian title of Paolo Rossi's important book *I Segni del Tempo*, now available to a wider audience in an English translation.

The objects grouped together here were also "signs of time" in another sense, as indications that the world might be far older than was generally thought. It is difficult for readers steeped in the time-scale of modern geology to recapture the threat that seemingly limitless time posed to men of science in the late seventeenth and early eighteenth centuries. The Copernican revolution and its consequences are familiar

from many historical discussions. But as Rossi notes, "there has been no such insistence, in the same way and with the same intensity, on other, no less decisive changes" — notably our perception of time. In the late seventeenth century, the direct and best testimony on the early ages of the world's history — the Bible — rendered an estimate of some few thousand years since the beginning not only intellectually respectable, but almost inescapable. By the end of the eighteenth, scholars looked down into what Buffon called "the dark abyss of time", and in advanced circles an age for the Earth of millions of years had become a distinct possibility. The Bible was being replaced as a secure source of knowledge by broken fragments of strata, old legends and cryptic languages. The old authority had provided at least the possibility of a single coherent narrative for the history of the world; the new ones could only be deciphered piecemeal and with difficulty.

As Rossi demonstrates, this change held major consequences for the organization of knowledge. In Genesis, the world commenced in the space of a few days. This meant that the history of the Earth and the history of man could be studied as one subject, for the two histories were almost precisely coextensive. This is a basic point, but one all too often set aside by those concerned with finding the roots of modern scientific disciplines. For instance, Robert Hooke, John Woodward, William Whiston and Thomas Burnet are familiar names in the history of geology. Rossi shows that their writings need to be read alongside the works of less familiar authors such as William Warburton, Isaac de La Peyrère and Martino Martini. Some of these men are well known in accounts of the origins of other disciplines (Egyptology, for example), while some have fallen into obscurity. By rescuing them from oblivion and reconstructing the character of their original concerns, Rossi traces the

contours of a fascinating and alien map of knowledge.

The Dark Abyss of Time looks particularly closely at those who maintained the authority of scripture against Hobbits, atheists, sceptics, pre-Adamites and others ready to use new empirical findings to undermine religious certainty and the social fabric. Discussion of these "defence mechanisms" is divided into three parts, devoted respectively to Earth history, human history, and barbarism and language. Rossi is especially concerned to show how each of these topics became entwined in the making of a single major text in early modern intellectual history, Giambattista Vico's *Nuova Scienza*, published in its final form in 1744. Usually hailed as a founding figure of historicism and a precursor of Benedetto Croce, Vico appears here in dialogue with contemporary figures and themes. With scholarly finesse, Rossi depicts the author of the *Nuovo Scienza* picking his way "across the scorching hot, difficult terrain" of controversies about the origin of man and the Earth. Far from being an isolated forerunner, Vico can be seen charting a new path through territory that had been an intellectual battleground for decades.

This is an impressively researched book, but one with very few concessions to students or non-specialist readers. (A good introduction is available in the first two chapters of Martin J. S. Rudwick's *Meaning of Fossils*, recently reissued in paperback.) Some of the difficulties are simply a matter of style, especially as the analysis has a tendency to loose momentum in lengthy summaries of cosmological theories. Individual chapters are not clearly connected, and have something of the character of separate essays. Moreover, the main thrust of the argument is occasionally obscured by traces of the forward-looking disciplinary history that most of the book rightly questions. Chapters on Kelvin and the age of the Earth problem (and indeed, those on Buffon and James Hutton) seem especially out of place. The preface makes the real point of the book clear.

While placing Vico in a fresh perspective, Rossi has made a major foray into a vast and unexplored territory in the literature of early modern science, pointing a way that others will want to follow. At first glance, his book may seem to deal with a variety of subjects, many not obviously relevant to the history of science. As a result, there is a real danger that readers concerned with the development of a single discipline will turn only to those chapters that immediately interest them. Resist the temptation, for such an approach perpetuates precisely those intellectual blinkers that so frequently limit an appreciation of other ways of seeing the world. □

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Oblateness of the Sun in 1983 and relativity

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Measurements of the solar oblateness obtained during 1983 from Mount Wilson, California, yield a value with an upper bound only half of that observed in 1966. This difference may support the conjecture that the solar quadrupole moment slowly oscillates. A knowledge of the character of such an oscillation, if it occurs, would be needed to test Einstein's relativity theory using Mercury's orbital motion.

THE motion of the perihelion of Mercury's orbit provided the first and potentially one of the most important tests of Einstein's general relativity theory, but because of uncertainty concerning the solar quadrupole moment, this test has long been in doubt. In 1966, a measurement of the solar oblateness^{1,2} gave a value³ of $\Delta r = r_{\text{eq}} - r_{\text{p}} = 41.9 \pm 3.3$ arc ms (or 30.6 ± 2.4 km) for the excess equatorial radius of the Sun, that is the fractional equatorial excess of $\Delta r/r = 4.34 \times 10^{-5} \pm 0.34 \times 10^{-5}$. This seemed to imply a solar quadrupole moment large enough to cause a significant rotation of Mercury's perihelion.

This oblateness was first interpreted¹ as the effect of a rapidly rotating solar core, but the indication that the distortion was rotating rigidly across the Sun's surface with a 12.38 ± 0.10 day sidereal period^{3,4} cast doubt on this interpretation. It seemed more likely that the distortion resulted from a strong magnetic field, primarily toroidal, in the solar core rotating with this period⁵⁻⁷. Such an explanation could permit long-period toroidal oscillations of the core. It has been speculated that the sunspot cycle might result from a magnetic field generated by such an oscillation⁶. If so, the solar oblateness might oscillate, varying with the sunspot cycle. This test of general relativity must take such a variation into account, if it occurs. There is evidence that the solar cycle is associated with a precisely tuned oscillator deep in the Sun^{6,8}.

In 1975 a substantially smaller solar oblateness was reported⁹ and Hill *et al.*¹⁰ have suggested recently that the spectrum of solar oscillations they have found implies a rapidly rotating solar core and a significant quadrupole moment.

More recently, observations of the 5-min oscillations have given a core rotation rate roughly twice that of the solar surface, a core rotation which is too slow to yield a quadrupole moment significant enough for the relativity test¹¹.

1983 observations

During 10 April to 27 September 1983, measurements of the Sun's shape were obtained (~ 8 h per day) by one of us (K.G.L.) on Mount Wilson, California, with an improved version of the Princeton solar oblateness telescope which had been used in 1966 to measure the oblateness of the Sun². As in the earlier version of the telescope, the Sun's image is projected on one of three occulting disks of different diameter, exposing 9–20 arc s at the Sun's limb. This exposed limb is scanned at ~ 10 Hz using a rotating wheel containing two diametrically opposed 2° apertures to look for the variation in the light flux with angular position. An ellipticity of the solar disk would cause a second harmonic variation of the light flux with angular position on the limb. But at least part of such a variation could result from a 'brightness signal', a variation of the brightness of the solar disk with angular position.

Mount Wilson is better than the Princeton site in one important aspect: it yields a much greater fraction of days with a clear

sky. But in another aspect it seems to be less satisfactory: at the Princeton site the flat grassy terrain apparently resulted in a symmetry in the locally induced atmospheric distortion of the Sun that simplified the data analysis, this symmetry was not present in the Mount Wilson data, probably because of the mountainous terrain.

The principal improvements made to the instrument are (1) in the modified instrument, the light flux from the partially occulted Sun is averaged in 128 channels 1.41° wide (in 1966, only the second harmonic sine and cosine distributions of the light flux were measured). (2) The servo system centering the Sun's image on the occulting disk is based on a new, significantly improved, technique. It includes both fast and slow (integrating) servo loops. (3) The two diametrically opposed scanning apertures are the same size. This eliminates from the total light flux, in first order, signals induced by small solar-image displacements. (4) Measurements are made at eight angular positions of the vertically mounted telescope, eliminating telescope errors up to the 16th harmonic. (5) The measurements are made simultaneously in two wide colour bands centred at 'green', $0.53 \mu\text{m}$, and at 'red', $0.80 \mu\text{m}$. These bands are, respectively, 0.08 and $0.30 \mu\text{m}$ wide.

The light flux measured at 128 angular positions permits the direct detection and rejection of channels containing the signals from substantial facular patches. A channel is rejected (for the whole day and for all three disks) if the daily mean of the excess signal in the channel exceeds 3σ . (In 1966, corrections for faculae were made indirectly, by least squares, assuming that the facular signal was proportional to the area of a facular patch.)

Using the 1983 data, the new instrument has permitted a direct determination of the facular contrast near the solar limb by Libbrecht and Kuhn¹². The variation of the facular signal with the amount of exposed limb is consistent with the 1966 results¹³. Evidence for a fourth harmonic variation of the effective temperature about the solar limb with an amplitude of $0.6^\circ \pm 0.1^\circ$ has been found in the 1983 data by Kuhn *et al.*¹⁴.

As in 1966, the second harmonic corrections for astigmatic mirror errors are made by combining data obtained with the mirrors in four different combinations of positions, the primary and secondary mirrors each being in two positions 90° apart, and then including the four astigmatic mirror parameters in the least-square fits to the data.

Geometrical optics provide an inadequate description of the mirror-induced distortion of the Sun's image: for the distortion parameters obtained from the least-square fits depend sensitively on both the colour and the amount of exposed limb. It seems likely that the mirror distortion is a diffraction effect resulting from $\lambda/20$ roughness of the mirror surfaces on a scale of a few centimetres.

After the above correction is made, a small residual error remains in the correction for the primary mirror. It is eliminated by assuming that the mirror distortion is a function of the angle of incidence of sunlight on the mirror and including this type

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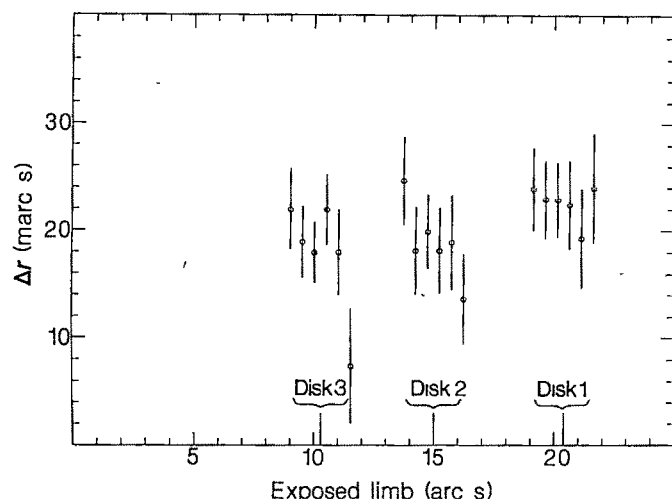


Fig. 1 Green solar oblateness. The values of Δr (in arc ms) derived from equation (3) are plotted for both colours and each solar-hour angle bin as a function of the exposed solar limb (in arc s).

After eliminating the channels contaminated by identifiable facular brightness signals, the second harmonic sine and cosine components of the light flux are obtained by least squares from the data, with the angular coordinate chosen to be zero at the terrestrial north point on the solar disk (not the solar pole). After dividing by the solar brightness at the edge of the occulting disk (and reversing the sign of the cosine component), twice the amplitudes of the sine and cosine components are expressed in arc ms and are designated Δr_d , the 'diagonal' component, and Δr_v , the 'vertical' component, of the solar ellipticity.

These two ellipticity components, corrected for mirror errors, are also corrected for the distortion induced by the refraction of a laminar atmosphere and are averaged for each day in six solar-hour angle bins, 20° wide, ranging from mean solar-hour angles of -50° to $+50^\circ$. In the discussion below, these six bins are designated $h = 1, 2, \dots, 6$, respectively.

The three occulting disks are designated $d = 1, 2$ and 3 and expose annuli at the solar limb with the seasonal mean widths for red of $\delta R = 18.1, 13.2$ and 9.2 arc s respectively and for green $\delta R = 20.4, 15.0$ and 10.3 arc s. These annular widths are defined by $\delta R = F/I$ where F is the light flux per degree past the disk and I is the solar brightness at the edge of the occulting disk expressed in flux units per deg arc s.

For an oblate Sun with oblateness $\Delta r = (r_{eq} - r_p)$, the solar oblateness contributes $\Delta r \sin 2P$ and $\Delta r \cos 2P$, respectively, to the diagonal and vertical components of the ellipticity. Here P is the counterclockwise position angle of the solar north pole relative to the north point on the solar disk and ranges from -25° to 25° during the observational period.

Atmospheric distortion

The distortion of the Sun's image by the Earth's atmosphere, assumed to be uniformly laminar, is not the only atmospheric distortion of the Sun. The temperature inhomogeneity of the air, primarily near the observatory, also distorts the Sun. This leads to the displacement of the time-averaged position of any stellar image and the spreading of the point image into a distorted seeing disk. We are concerned here with the time-averaged position and ellipticity of the seeing disk, the two dominant contributions to the elliptical distortion of the solar image having their origin in this local temperature inhomogeneity. They are called, respectively, the 'cylindrical lens' distortion and the 'anisotropic seeing' distortion². These are the two lowest order (dominant) terms in the distortion obtained from an expansion of the solar brightness, $I(x)$, in moments of the distorted seeing disk.

The cylindrical lens distortion is independent of the limb

Table 1 Results of least-squares fits, equation (3)

Disk	Bin	Δr	D	Δr	D
1	1	26.3	33.6	23.9	37.0
1	2	23.6	15.1	22.9	12.9
1	3	22.6	3.5	22.9	-0.8
1	4	22.5	-1.4	22.4	-4.3
1	5	18.8	-7.0	19.3	-7.2
1	6	27.0	-2.5	24.0	1.8
2	1	24.4	32.4	24.7	35.0
2	2	16.6	10.8	18.1	5.3
2	3	17.8	8.4	19.9	-0.1
2	4	17.6	3.7	18.1	-3.9
2	5	20.3	2.7	18.9	-1.7
2	6	17.6	3.0	13.6	1.3
3	1	27.1	23.8	21.9	33.4
3	2	19.8	7.6	18.9	10.3
3	3	13.5	2.0	17.9	1.6
3	4	17.2	4.4	21.9	1.5
3	5	16.2	9.7	18.0	3.0
3	6	12.9	19.4	7.4	12.3

distortion is proportional to the logarithmic radial derivative of the solar brightness at the edge of the occulting disk. For disks $d = 1, 2$ and 3 this is respectively for the red colour band:

$$S(d) = 1, 1.31 \text{ and } 1.84 \quad (1)$$

normalized to unity for disk number 1. For green the corresponding values are:

$$S(d) = 1, 1.28 \text{ and } 1.75 \quad (2)$$

respectively. These values are obtained from limb-darkening curves for these colours¹⁵.

For the vertical component, the atmospheric contribution to the apparent solar ellipticity is not statistically separable from the true solar oblateness, for $\cos 2P$ varies relatively little through the observing period. Another quite different difficulty with the vertical component of the data concerns the off-axis astigmatic error of the primary mirror. This cannot be determined from the mirror cycling, but it affects only the vertical component of the distortion. Because of these difficulties, the analysis discussed below is based only on the diagonal component of the ellipticity.

The contribution of the solar oblateness to the diagonal component of the solar ellipticity, $\Delta r \sin 2P$, can be determined by least squares using the known seasonal variation of $\sin 2P$. This determination of Δr can be made for each of the six solar-hour angle bins for each of the three disks and for each of the two colours by fitting

$$\Delta r \sin 2P + D \rightarrow \Delta r_d \quad (3)$$

Here $D(h, d)$ is interpreted as the seasonal mean of the local atmospheric contribution to Δr_d . To interpret Δr as the solar oblateness may be premature. There are two reasons for caution. First, there may be a fortuitous $\sin 2P$ seasonal variation in the atmospheric distortion of the Sun. Second, the excess light flux in the equatorial part of the Sun may be due, at least in part, to an excess solar brightness near the equator.

The results of the least-square fits, equation (3), to the diagonal component of the ellipticity are given in Table 1. The derived values of the Δr are plotted in Fig. 1 as a function of exposed limb for each bin. Here, and in Table 1, Δr and D are expressed in arc ms. The means of the standard errors of Δr are 3.6 and 4.0 arc ms for red and green, respectively. For D the mean errors are 1.9 and 2.0 arc ms respectively.

In the 36 fits, equation (3), 3σ deviations are much more numerous than the 6 expected with a normal distribution of errors. It is found that the 30 excess large deviations all occur

may be abnormal and that they should be eliminated from the original list of 131 days with data. With the criterion that there are 2 or more 3σ deviations present, the 6 days with day numbers 98, 99, 129, 137, 159 and 175 are eliminated from the fit, equation (3), for the results given in Table 1 and in Fig. 1.

Note that the large variation of the atmospheric distortion D (as a function of h for fixed d) is not seen in Δr . Thus, it is unlikely that the atmospheric distortion has a fortuitous $\sin 2P$ dependence on time. The question of a 'brightness signal' contribution to the signal is discussed below.

For the hour angle bins $h=1$ and 6, the Sun is fairly low in the sky and the atmospheric distortion is large. (Note the large values of $D(1, d)$ in Table 1.) The data in bins 1 and 6 may introduce both a systematic statistical bias and excess noise. In the analysis below, these bins will be dropped, but the analysis will also be carried out with these bins included.

We assume that the locally induced atmospheric distortion of the Sun, $D(h, d)$, consists of the sum of two parts, the cylindrical lens distortion, $C(h)$, and the anisotropic seeing distortion, $S(d)A(h)$, where $S(d)$ is given by equations (1) or (2). The least-square fit

$$\Delta r(d) \sin 2P + C(h) + S'(d)A(h) \rightarrow \Delta r_d \quad (4)$$

combines data from all three disks and is computed for each of the two colours. The oblateness $\Delta r(d)$ is a function of disk number to allow for the possibility of a contribution from a 'brightness signal'.

To test the atmospheric distortion model, $S'(d)$ is included with $C(h)$, $A(h)$ and Δr to be varied in the least-squares fits. The results obtained for the ratio $(S'(3)-1)/(S'(2)-1)$ are 2.77 ± 0.35 and 4.1 ± 1.1 respectively for the red and green signals and are in satisfactory agreement with the ratio 2.71 obtained from equations (1) and (2). Consequently, these equations are used when computing the corrections for atmospheric distortion, using equation (4).

The results obtained from equation (4) for red are $\Delta r(d) = 21.7 \pm 1.7$, 18.3 ± 1.7 and 16.6 ± 1.7 for $d=1, 2$ and 3, and for green, 22.0 ± 1.9 , 18.8 ± 1.9 and 19.2 ± 1.9 arc ms. Note that, independently of the colour and the disk number, the six values of Δr do not differ significantly from their mean. For $C(h)$ and $A(h)$ the results are given in Table 2. The corresponding mean

Table 2 Atmospheric distortion (arc ms)

	h	2	3	4	5
Red	$C(h)$	23.0 ± 4.1	8.8 ± 4.1	-6.3 ± 4.2	-24.9 ± 4.3
Red	$A(h)$	-8.6 ± 2.9	-3.0 ± 2.9	6.2 ± 3.0	19.3 ± 3.0
Green	$C(h)$	12.1 ± 4.9	-4.0 ± 4.9	-13.4 ± 5.0	-19.8 ± 5.1
Green	$A(h)$	-1.9 ± 3.5	3.2 ± 3.5	8.3 ± 3.6	13.2 ± 3.7

estimated post-fit errors in the data points are for red, 18.8 ms, and for green, 20.5. The number of data points (20° binned means) is 1,359.

The four (or six) bins of data for each colour and disk, corrected for local atmospheric distortion using the results in Table 2, are averaged to form sets of daily averages. Only days for which data exist for all three disks are kept, a total of 121 days for the daily means of bins $h=2$ to 4.

Brightness signal

To compute the brightness signal contribution to the apparent solar oblateness, we assume that the green brightness signals are greater than the red by a factor λ , to be determined. For excess light flux resulting from faculae, we measure $\lambda = 1.6$ and for a small change in the photospheric temperature we expect a value of 1.5.

The daily mean values of the diagonal component of the solar

parts, a shape signal S and a brightness signal λB where $\lambda = 1$ for red.

The mean value M and the semi-difference D of the green and red signals are:

$$M = S + 0.5(\lambda + 1)B \quad (5)$$

$$D = 0.5(\lambda - 1)B$$

The colour difference D provides a measure of the brightness signal for each of the three disks.

The red and green day-to-day fluctuations (noise) are mainly in the shape signals. The variances of M , computed from the residuals after removing the fitted ellipticity, are 9–30 times as great as those of D . The 3×3 covariance matrix of D apparently requires two separate brightness signals common to all three disks and a low noise level for disks 1 and 2. The brightness signal is strongest in D_1 (disk 1) which is adopted as one of the 2 signals. The second $D' = D_2 - 0.70 D_1$ is orthogonal to D_1 .

The least-squares fit

$$\Delta r \sin 2P + c_1 D_1 + c_2 D' \rightarrow M \quad (6)$$

is used to fit simultaneously for the oblateness and the two components of the brightness signal. The results are given (in arc ms) in Table 3. The last column is the estimated standard error of the daily means of the solar ellipticity, M .

Table 3 Least-squares fits for the solar oblateness and brightness

Disk	Δr	C_1	C_2	σ
1	20.9 ± 1.9	2.80 ± 0.38	1.49 ± 0.78	10.36
2	18.5 ± 2.0	1.73 ± 0.41	0.69 ± 0.84	11.16
3	18.3 ± 1.8	1.20 ± 0.36	0.22 ± 0.75	9.87

The first brightness term is statistically significant for all three disks, but it contributes very little to the apparent oblateness of the Sun. This brightness varies on a timescale of 1 day but is not apparently concentrated at the equator or pole.

In Table 3, the mean of the three values of Δr is 19.2 ± 1.4 . The deviations of the three values of Δr from the mean are not statistically significant and these deviations provide no evidence for the existence of some additional (unmodelled) brightness signal.

The factor λ in equation (5) can be evaluated as $\lambda = (c_1 + 1)/(c_1 - 1)$ from the first line of Table 3, neglecting D' . This gives the result $\lambda = 2.1 \pm 0.3$, (-0.2). If the same fit, equation (6), is made using the daily means of the solar ellipticity which include the bins 1 and 6, the results for Δr are: 23.7 ± 1.8 , 19.2 ± 1.8 and 18.7 ± 1.8 ms.

The above formal standard deviation of the mean solar oblateness, 19.2 ± 1.4 arc ms, is not a realistic measure of the error. There could be a seasonal variation in the atmospheric distortion that would add to or subtract from the apparent oblateness, perhaps 1–3 arc-ms. A much larger variation seems to be precluded by the absence of a marked $\sin 2P$ variation in the various weather parameters and in the declination of the Sun.

A more serious problem is the possible existence of another, colour-independent, brightness signal, for which $\lambda = 1$. Such a signal would escape the above analysis. There is no evidence for the existence of such a signal, for the oblateness is seen to be substantially independent of disk number. Nonetheless, a small signal of this type could exist and increase the error of the oblateness determination.

To include the effect of such a signal, we use the least-squares fit

$$\Delta r \sin 2P + BE(d) + c_1(d)D_1 + c_2(d)D' \rightarrow M \quad (7)$$

Here the second term is the new brightness signal and the third and fourth terms are those previously discussed. We assume that the colour-independent brightness signal is proportional to the exposed limb and this factor is $E(d) = 1, 0.73$ and 0.50 . The

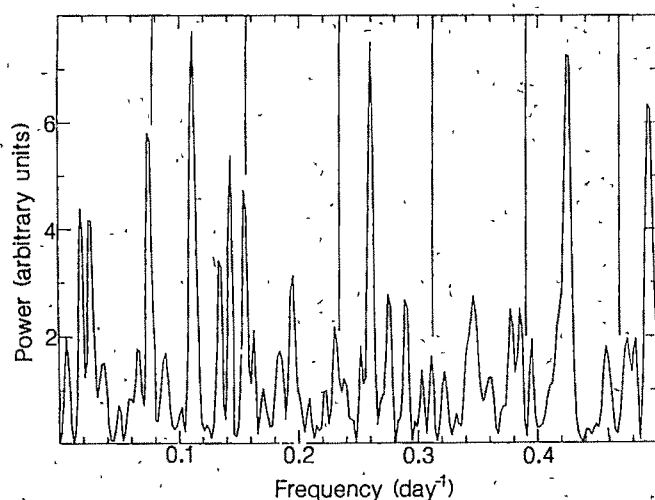


Fig. 2 The power spectrum of the residuals of M_2 (disk 3), obtained by removing the fitted value of $\Delta r \sin 2P$, from M . The vertical lines give the frequencies of the first six harmonics of the 1966 frequency, 0.07804 per day.

It gives the results $\Delta r = 15.4 \pm 4.1$ and $B = 5.2 \pm 5.3$ arc ms. Note that the brightness signal B is not statistically significant and that the change in the oblateness is only 1 standard deviation (s.d.). Nonetheless, the inclusion of this additional brightness term increases the error of the oblateness substantially, and the oblateness could be low enough to make the solar quadrupole moment negligibly small. If the daily means of the solar ellipticity which include the bins $h=1$ and 6 are used, the results $\Delta r = 13.0 \pm 3.9$ and $B = 10.2 \pm 5.0$ are obtained.

12.38-day rotational period

As noted above, the 1966 observations of the solar ellipticity were best interpreted as the effect of a solar distortion rotating rigidly over the surface with a (sidereal) period of 12.38 days. The first three harmonics of the synodic frequency, 0.07804 ± 0.0006 per day, appeared in the power spectrum of the residuals (after subtracting the fitted oblateness term). This periodic signal is much weaker in the 1983 data, if it is present at all.

The power spectrum of the residuals of M_2 (disk 3) is shown in Fig. 2. The vertical lines give the frequencies of the first six harmonics of the 1966 frequency, 0.07804. The first two harmonics may be present in the 1983 data but these two frequencies are not the two most prominent peaks in the power spectrum. The first harmonic is also present in M for the other two disks, where the noise level is higher, and in D_1 where the amplitude is 1.0 ms, but the error is only 0.3 ms.

A least-squares fit to the above two peaks for a rigidly rotating distortion yields a rotational frequency $f = 0.0792 \pm 0.0005$ per day, in good agreement with the frequency obtained from the 1966 data³. However, the amplitudes of the first two harmonics of the fitted function are only one-fifth and one-half of those obtained from the 1966 data.

In the power spectrum, there are four strong peaks for which we have no explanation. Could some of the other peaks be aliases of these four peaks (arising from the complexities of the window function due to missed observational days)? This is unlikely, for the aliases are very weak, with powers only a few per cent of the powers of the main peaks in the spectrum.

Despite the occurrence of the harmonics of $f = 0.0792 \text{ yr}^{-1}$ in the power spectrum, we do not believe that the existence of the 12.4-day rotational period in the data has been demonstrated. A much stronger case for the existence of this rotational signal was made using the 1966 data^{4,16}.

Conclusions

Assuming that the brightness signal is colour dependent, the value of the oblateness of the Sun obtained in 1983 is 19.2 ± 1.4 arc ms, where the error is only a formal s.d. A seasonal variation of the atmospheric distortion (in the form $\sin 2P$) might occur. It could have either sign and contribute an error to the oblateness determination, perhaps as great as ± 3 ms. If there should also be a colour-independent brightness signal proportional to the exposed limb (in addition to the colour-dependent one) the value 15.4 ± 4.1 would be obtained for the oblateness, where the error is again only formal. For both of these possibilities the 1983 result for the oblateness is $\ll 42 \pm 2$ ms obtained in 1966.

Unless there is some support for the existence of a colour-independent brightness signal, it seems reasonable to adopt 19.2 ± 1.4 ms for the oblateness, with the proviso that a more believable error might be ± 4 ms. Of this oblateness, 7.8 ms results from surface rotation (a centrifugal distortion of surface layers)¹⁷. The remainder, 11.4 ms (or $\Delta r/r = 1.2 \times 10^{-5}$), is associated with a quadrupole moment $J_2 = (2/3)(\Delta r/r) = 7.8 \times 10^{-6}$ which is only one-third of the value obtained from the 1966 observations.

The 1983 results seem to be compatible with the conjecture that the Sun has an oscillating quadrupole moment, but an extended series of observations would be needed to confirm this conclusion. The implications of the solar oblateness for Einstein's theory of gravitation will be unclear until the solar quadrupole moment and its possible variation with time are measured. The implications of an oscillating solar core for the solar cycle, and for the generation of solar magnetic fields, could be profound.

The classical astronomical test of Einstein's theory derived from the excess rotation of the perihelion of Mercury's orbit requires a knowledge of J_2 and its variation with time. If variation of J_2 were periodic with the sunspot cycle, a 22-yr mean would be adequate. More recent planetary radar observations of Mercury's motion can use time averages on a decade timescale. A Mercury satellite orbiter could reduce this timescale markedly and permit an observation of the time variation of the perihelion rotation. If the time variation of J_2 were also known, this would permit an accurate test of this relativistic effect on a short timescale¹⁸.

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Amino-acid sequence of the catalytic subunit of the $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ deduced from a complementary DNA

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We have isolated and characterized a complementary DNA for the catalytic subunit of the sheep kidney sodium/potassium-dependent ATPase. The 1,016-amino-acid protein seems to have eight transmembrane domains. The apparent ouabain binding site is located at the extracellular junction of two transmembrane domains and is linked to the phosphorylation site by a 60-amino-acid conserved sequence that may be a major channel for energy transduction.

It is known that the sodium/potassium-dependent ATPase $[(\text{Na}^+ + \text{K}^+)\text{ATPase}]$ is an integral membrane protein that directly couples the hydrolysis of ATP to the vectorial transport of Na^+ and K^+ across the plasma membrane. This in turn produces the electrochemical gradient of Na and K which is the primary source of energy for the active transport of various nutrients¹, the action potentials of excitable tissues such as muscle and nerve² and the regulation of cell volume³. The enzyme consists of a large catalytic subunit (α), with a relative molecular mass (M_r) of 110,000, and a smaller glycoprotein subunit (β), of unknown function, with an M_r of ~55,000 (ref. 4). The ATP-binding site⁵ and the phosphorylation site⁶ are both located on the cytoplasmic side of the α -subunit and a binding site for cardiac glycosides such as digoxin and ouabain is located on the extracellular side⁷. There is much interest in the location and structure of the ouabain binding site because it is thought to be the pharmacological receptor site for the digitalis class of drugs⁷⁻¹¹.

Considerable effort has been directed at determining the molecular mechanism of active cation transport. The catalytic subunit can exist in at least two conformational states and it is apparently during the transition between these states that Na^+ and K^+ traverse the membrane^{12,13}. This conformational transition is extensive and seems to involve at least 80 amino acids which undergo a transition between α -helix and β -sheet¹⁴ during each cycle of phosphorylation and dephosphorylation. Because the binding of Na^+ and K^+ , cardiac glycosides and ATP all influence the conformational state of the enzyme¹³, there seems to be communication throughout the molecule, involving cytoplasmic, transmembrane and extracellular domains. In addition to the $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ there are several other eukaryotic plasma membrane proteins involved in primary active transport. These include the Ca^{2+} -ATPase¹⁵, the $(\text{H}^+ + \text{K}^+)\text{ATPase}$ ¹⁶, and an H^+ -ATPase found in fungi¹⁷. The available data suggest similarities in their structures and enzymatic mechanisms and there is evidence that they have evolved from a common ancestor¹⁸. Structural comparisons of these transport ATPases may help to elucidate their function.

Here we describe the isolation and characterization of a complementary DNA clone containing the entire coding region of the messenger RNA for the catalytic subunit of the sheep kidney $(\text{Na}^+ + \text{K}^+)\text{ATPase}$. The complete amino-acid sequence has been deduced and a comparison with the Ca^{2+} -ATPase sequence reveals extensive regions of homology. This and some other comparisons have allowed us to identify several possible functional domains, including a conserved sequence of ~60 amino acids which is likely to be a major component of the energy transduction system. This sequence spans the membrane and is bounded at one end by the phosphorylation site and at the other by the apparent ouabain binding site.

Isolation of cDNA clones

Sixteen tryptic peptides of the sheep kidney $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ α -subunit have been characterized previously¹⁹ and two of these peptides, T14 and T16, were used to derive oligonucleotide hybridization probes. T14, located at the ATP-binding site, contains the sequence Met-Lys-Gly-Ala-Pro and T16 contains the sequence Val-Phe-Gln-Ala-Asn-Gln-Asp. A 14-base probe, 5' G-G-(A,G,T,C)-G-C-(A,G,T,C)-C-C-(C,T)-T-T-C-A-T 3', which was a mix of 32 oligonucleotides, and a 20-base probe, 5'-T-C-(T,C)-T-G(A,G)-T-T(A,G,T,C)-G-C(T,C)-T-G-(A,G)-A-(A,G,T,C)-A-C3', which was a mix of 256 oligonucleotides, were derived from T14 and T16, respectively. A library of 50,000 colonies constructed with size-selected cDNA prepared from sheep kidney poly(A)⁺ RNA was screened separately with each probe. Twelve colonies gave signals with both oligonucleotides and plasmid DNA from these clones was isolated and analysed by restriction mapping and blot hybridization analysis.

The size of the cDNA inserts ranged from 2.30 to 3.65 kilobases (kb) (data not shown). Eleven of the twelve clones had a 1.85-kb *Pst*I fragment, which corresponds to the 3' end of the mRNA, and a variable *Pst*I fragment ranging in size from 0.45 to 1.8 kb, which corresponds to the 5' end of the mRNA. In each case a 0.44-kb *Bam*HI restriction fragment gave strong signals with both oligonucleotides when analysed by blot hybridization. To confirm the identity of these clones we first determined the nucleotide sequence of αNKA1 in the region containing homology to the hybridization probes (see Fig. 1a for the restriction map of αNKA1 and the sequencing strategy). The nucleotide sequences encoding T14, T16 and several other known tryptic peptides of the sheep enzyme were identified. All 16 of the known tryptic peptides have been identified in the full sequence described below.

Nucleotide and amino-acid sequences

The complete nucleotide sequence for the largest clone, αNKA1 , and the deduced amino-acid sequence are shown in Fig. 1b. It contains 254 bases of 5'-untranslated sequence, a 3,063-base open reading frame and 340 bases of 3'-untranslated sequence. An additional 10 bases of 5'-untranslated sequence were determined from clone αNKA2 and are included in Fig. 1b. The 3'-untranslated region contains the canonical polyadenylation signal²⁰ 23 bases upstream from the poly(A) tract. The position of the poly(A) tract was confirmed by sequencing the 3' end of αNKA3 , which contains a 14-residue poly(A) tract in this position rather than the three A residues seen in αNKA1 .

A striking feature of the 5'-untranslated region is the G + C-richness (72%) with an abundance of CpG dinucleotides, which are under-represented in the vertebrate genome as a



Fig. 1 Sheep kidney ($\text{Na}^+ + \text{K}^+$)ATPase α -subunit cDNA. **a**, Restriction map of clone pNKA1 and sequencing strategy. Only the restriction sites (P, *Pst*I; A, *Aua*I; Xh, *Xho*I; S, *Sall*; E, *Eco*RI; Xb, *Xba*I; B1, *Bgl*I; B, *Bam*HI; N, *Nco*I; BII, *Bgl*II) relevant to the sequencing strategy are shown. The extent and direction of sequencing are indicated by arrows (lower arrows, coding strand; upper arrows, noncoding strand). Hatched areas represent the 5'- and 3'-untranslated regions. **b**, Nucleotide sequence of clone pNKA1 and deduced amino-acid sequence. The first 10 nucleotides at the 5' end were determined from clone pNKA2 which extended slightly beyond the 5' end of pNKA1. Amino acids are numbered starting at the N-terminal Gly of the mature protein and are preceded by five amino acids occurring in the primary translation product. The eight major hydrophobic regions (H1-H8) are underlined. Brackets indicate the putative phosphorylation site (P), centred around Asp 369, and a portion of the ATP-binding site region (A), which includes Lys 501.

Methods. Poly(A)⁺RNA was isolated^{46,47} from sheep kidney. cDNA was synthesized by the procedure of Gubler and Hoffman⁴⁸, size-selected by electrophoresis in agarose and capture of the 2,100–4,500-base-pair fraction on NA45 paper (Schleicher & Schuell), tailed with dCTP and annealed⁴⁹ with G-tailed *Pst*I-cut pBR322. *E. coli* RRI cells were transformed with 60 ng of cDNA and grown on agar plates containing tetracycline. Colonies were transferred to nitrocellulose master filters which were used to prepare two replicas⁵⁰ on Zeta Bind membranes (AMF Cuno). Following additional growth on tetracycline and amplification on chloramphenicol, the filters were processed by the procedure of Geyer and Johnson⁵¹. Filters were hybridized successively with two oligonucleotide probes (see text for composition of probes) which were 5' end labelled using *T*₄ polynucleotide kinase and [γ -³²P]ATP. Conditions for hybridization, washing and autoradiography were basically as described by Boyd *et al.*⁵². DNA sequencing was performed by the chemical cleavage method of Maxam and Gilbert⁵³. Restriction fragments were labelled either at the 5' end using [γ -³²P]ATP and *T*₄ polynucleotide kinase, or at the 3' end using the Klenow fragment of DNA polymerase I and [α -³²P]dNTPs.

whole²¹. The phenomenon of CpG suppression is attributed to the effects of methylation and subsequent mutation that causes a decrease in CpG content and a corresponding increase in the number of TpG and CpA dinucleotides²¹. In the case of the ($\text{Na}^+ + \text{K}^+$)ATPase mRNA, there is no apparent CpG suppression in the 5'-untranslated region; the CpG content is 90% of the value expected based on nucleotide composition, whereas the TpG+CpA content is 87% of the expected value. However,

CpG suppression is apparent in the coding region, where the CpG content was 46% of the expected value against 135% for TpG+CpA. Recent evidence suggests that G+C-richness may be a common feature of the 5' regions of housekeeping genes^{22,23} and the present results support that view. As suggested by Bird²⁴, it may be useful to consider an evolutionary perspective when assessing the significance of these G+C-rich tracts. As these regions have escaped CpG suppression their functional role

a

NKA 173 INAEVGVGLVGVKGGORIPADLRISANGC--KVDNSSLTGES 215 233 RNIAFFSTNCVEGTARGIVVYTGDRIVMGRIATLASGLEGGQTP 276
 CA 140 IKAXDIVPGDIVEIAVGDKVPADIRLTSIKSTTLAVDOSILTGES 184 205 KNMLFSGTNIAAGKAMGVVATGVNTEIGKIRDEMVAEQERTP 248

NKA 322 VAVNPÉGLLATVTVCLTITAKRMARKNCLVKNLEAVETLGSTSTICSDKTGTLTONRMTVAHM 384 499 VMKGAPÉRIILDRCCSILIHGKEQPLDEELK 528
 CA 304 VAAIPEGLPAVITTCALGTRMAKNNAIVRSLPSVETLGCTSVICSOKTGLTTLTNQMSVCRM 366 512 FVKGAPÉGVDRCTHIRVGSTKVPMTAGVK 541

NKA 575 NLCFVGLISMIDPPRAAVPDAVGKCRSAGIKVIMVTGDHPITAKAIAKGVGI 626 683 FARTSPQOKLIIVÉGCQRQGAIVAVTGDDGVNDSPALKKADI 723
 CA 589 NLTFVGCVMLOPPRIEVASSVKLRQAGIRVIMITGDNKGTAVAICRRIGI 640 675 FARVEPSHKSKIVEFLQSFDEITAMTGDGVNDAPALKKAEI 715

NKA 724 GVAMGIAGSDVSKQADMLDDNFASITVGVÉEGRLIFDNLKKSIAVTLTNSNIPÉ 779 807 TDMVPAISLAYEQAESDIMKRPANPQTDKLVNERLIS 844
 CA 716 GIAMG-SGTAVAKTASEMVLADDFSTIVAAVEEGRAIYNNMKQFIYLISSNVGE 770 798 TDGLPATALGFNPPDLIDMKNPPRNP---K---EPLIS 829

NKA 975 PLKPTWVFCAPPYSLLIFVYDÉVRKLIIR 1003
 CA 960 PLNVTQWLMVLKISLPVILMOETLKFFVAR 988

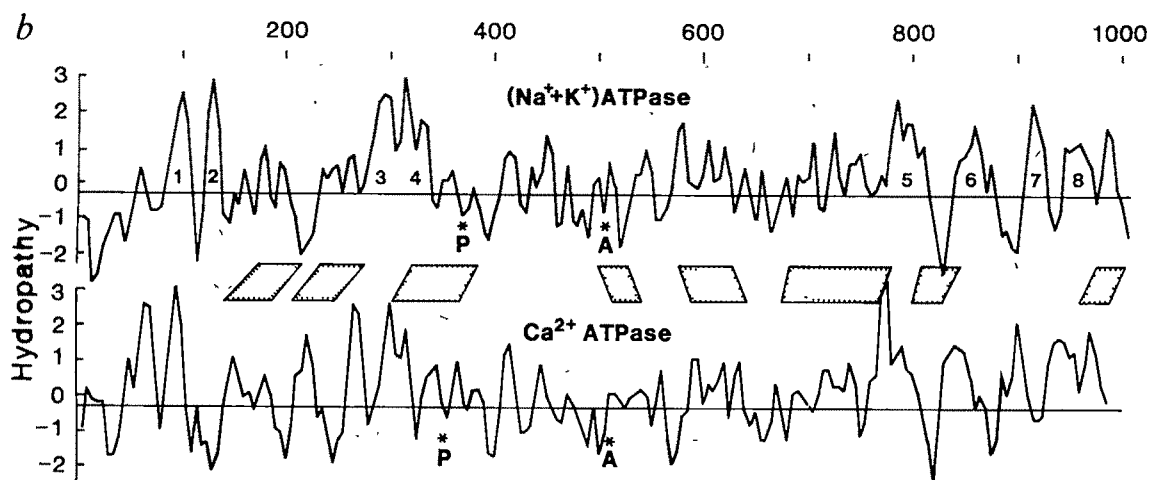


Fig. 2 Comparison of the amino-acid homology and hydropathy plots of the sheep kidney ($\text{Na}^+ + \text{K}^+$)ATPase α -subunit and the rabbit cardiac Ca^{2+} -ATPase. **a**, Amino-acid homology between the ($\text{Na}^+ + \text{K}^+$)ATPase (NKA) and the Ca^{2+} -ATPase (CA). The sequences compared are those with $>30\%$ homology. Homologous residues are indicated by an asterisk; gaps have been included to maximize homology. **b**, Hydropathy plots of the ($\text{Na}^+ + \text{K}^+$)ATPase (top panel) and Ca^{2+} -ATPase (bottom panel). Hydrophobic regions are above the x axis and hydrophilic regions below it. The parallellograms indicate the regions of homology shown in **a** and their relative positions along the peptide chains. The phosphorylation site is indicated by P and the ATP binding site by A. The numbers indicate the eight major hydrophobic regions of the ($\text{Na}^+ + \text{K}^+$)ATPase. Homology comparisons and hydropathy analyses were performed using the International Biotechnologies sequence analysis programs. The hydropathy program is based on the procedure of Kyte and Doolittle³¹.

may have little to do with methylation (which may be of recent evolutionary origin and confined to vertebrates²⁵) but instead could be part of an evolutionarily older regulatory process which operates in both vertebrates and invertebrates.

The identification of the translation initiation site is unambiguous as it is the first AUG codon; it is preceded by 264 bases of G+C-rich sequence and lies within the consensus sequence for initiation described by Kozak²⁶. The initiation methionine is located five amino acids upstream from the known N-terminal peptide of the protein¹⁹. Thus, the primary translation product does not contain a hydrophobic N-terminal signal peptide. Only a five-amino-acid sequence, Met-Gly-Lys-Gly-Val, is removed to yield the N-terminus of the mature protein, which has 1,016 amino acids and an M_r of 112,177. The C-terminal amino acids, Thr-Tyr-Tyr-COOH, are identical to those of the dog kidney enzyme²⁷.

Several considerations lead us to conclude that the Asp that is phosphorylated during the catalytic cycle²⁸ is at position 369. The most compelling evidence is its location within an extended region of homology with the phosphorylation site region of the Ca^{2+} -ATPase²⁹ (see below). Furthermore, the surrounding sequence, Cys-Ser-Asp-Lys (Fig. 1b), is consistent with that determined by Bastide *et al.*⁶ and lies within the region predicted on the basis of experiments using proteolytic digestion (reviewed in ref. 13). Several laboratories have identified a peptide containing a Lys residue which reacts with fluorescein isothiocyanate (FITC) and which forms a part of the ATP-binding site^{5,30}. This FITC peptide corresponds to residues 496–506 (Fig. 1b). The

reactive residue, Lys 501, is located 132 amino acids away from the phosphorylation site.

There are eight major hydrophobic sequences (H1–H8), ranging in size from 17 to 29 amino acids (see Figs 1b, 2b). Based on the hydropathy values of membrane-spanning segments analysed by Kyte and Doolittle³¹, it is likely that each traverses the membrane.

Homology with Ca^{2+} -ATPase

A comparison of the amino-acid sequence and hydropathy plot of the ($\text{Na}^+ + \text{K}^+$)ATPase α -subunit with those of the Ca^{2+} -ATPase³² is shown in Fig. 2. The overall structural organization of the ($\text{Na}^+ + \text{K}^+$)ATPase is strikingly similar to that of the Ca^{2+} -ATPase. The two proteins are virtually identical in size and in the location of their phosphorylation and ATP-binding sites and each of the eight large hydrophobic domains of the ($\text{Na}^+ + \text{K}^+$)ATPase has its counterpart in the Ca^{2+} -ATPase (Fig. 2b). Except for three regions where ≥ 20 amino acids seem to have been inserted into or deleted from one or the other protein, matching structural features occur throughout the peptide chains. These proteins have clearly evolved from a common ancestor.

Several major regions of amino-acid homology (Fig. 2a) are located between H2 and H3 and between H4 and H5. Experimental evidence, both for the ($\text{Na}^+ + \text{K}^+$)ATPase³³ and for the Ca^{2+} -ATPase¹⁵, suggests that these areas are on the cytoplasmic side of the membrane. The greatest homology is near the phosphorylation site. A 10-amino-acid sequence which includes Asn

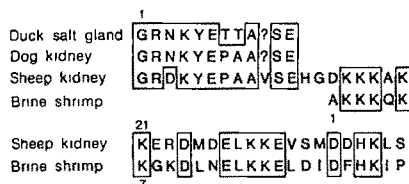


Fig. 3 Comparison of the N-terminal amino-acid sequences of the $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ α -subunit of several species. Identical residues are boxed. Duck salt gland data from ref. 35; dog kidney data from ref. 34; brine shrimp data from ref. 36.

369, is identical in the two proteins. The homology ends abruptly at Met 384, where the hydropathy values become hydrophilic for the $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ but are somewhat hydrophobic for the $\text{Ca}^{2+}\text{-ATPase}$. An insertion or deletion may have occurred here because the $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ has 32 fewer amino acids between the phosphorylation site and the ATP-binding site. There is only a small region of homology at the ATP-binding site but several longer regions of homology occur nearer the C-terminus, including an interesting hydrophilic domain at residues 807–844. If H5 and H6 span the membrane, this sequence of the $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ would be on the exterior of the cell, whereas for the $\text{Ca}^{2+}\text{-ATPase}$ it would be inside the sarcoplasmic reticulum.

There is no clear evidence regarding the location of the C-terminus for either the $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ or the $\text{Ca}^{2+}\text{-ATPase}$. However, as the last 20 amino acids of the $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ are fairly hydrophilic it is more likely to be located on the cytoplasmic side of the membrane. Also, a cytoplasmic location would be necessary if all four hydrophobic domains in the C-terminal half of the protein span the membrane. The hydropathy values³¹ of these segments and the corresponding segments of the $\text{Ca}^{2+}\text{-ATPase}$ suggest that this is the case.

N-terminal domains

The N-terminal region of the $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ is strongly hydrophilic and does not have a homologous counterpart in the $\text{Ca}^{2+}\text{-ATPase}$. If the first major hydrophobic domains of the two proteins are aligned it seems that the $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ has ~35 additional amino acids at its N-terminus (see Fig. 2b). Figure 3 shows a comparison of the N-terminal sequences of mammalian, avian and crustacean $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ α -subunits. The similarity between the sheep and dog kidney

enzymes³⁴ and that of the duck nasal salt gland³⁵ was noted previously but the crustacean (brine shrimp) sequences were thought to be very different³⁶. However, we find that this is not the case. Although the first 14 amino acids in the sheep enzyme are absent from the brine shrimp enzyme, there is considerable homology beyond that point, particularly the conservation of several lysine residues. The brine shrimp isoform that most closely resembles the sheep enzyme has 9 lysines among the first 26 amino acids³⁶. Eight of these residues are present in the sheep enzyme; an Arg is present at the remaining position. This is quite striking because the evolutionary divergence of these two species occurred over 400 Myr ago.

Several lines of evidence suggest that this region influences the cation-dependent equilibrium between the E1 and E2 conformational states³³. A conformation-dependent trypsin site ~20 amino acids from the N-terminus is cleaved rapidly in 10 mM NaCl, yielding an N-terminal Ala, but is inaccessible in KCl^{27,33}. The small fragment removed contains a histidine which may be involved in the cation-effect on the E1–E2 equilibrium^{33,37}. This trypsin site could be at Lys 18, as it is followed by an Ala and preceded by a His at position 13. The brine shrimp enzyme, which is exposed to a very different salt environment from that of mammals and birds, lacks the short sequence that contains His 13. An interesting possibility is that the N-terminal hydrophilic domain, which contains many charged residues including the highly conserved Lys residues (Fig. 3), controls the passage of Na^+ and K^+ ions to and from cation-binding sites on the cytoplasmic side of the protein. This possibility is consistent with the lack of homology in this region between the $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ and the $\text{Ca}^{2+}\text{-ATPase}$.

The N-terminus of the $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ ³⁸, like the $\text{Ca}^{2+}\text{-ATPase}$ ³⁹, is located on the cytoplasmic side of the membrane. As the region between H2 and H3 is also cytoplasmic, H1 and H2 probably span the membrane and the hydrophilic junction between them should be located on the extracellular side of the membrane. It has been suggested that transmembrane sequences in this region form part of an ion channel³³. The presence of several acidic residues between H1 and H2, which could serve as cation-binding sites on the extracellular surface, is consistent with this possibility. Also, Ca^{2+} -ionophore activity has been demonstrated for the corresponding region of the $\text{Ca}^{2+}\text{-ATPase}$ ⁴⁰.

Energy transduction region

One of the most interesting sections of the $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ is the region which contains the phosphorylation site and hydrophobic domains H3 and H4 (Fig. 2b). A proteolytic fragment

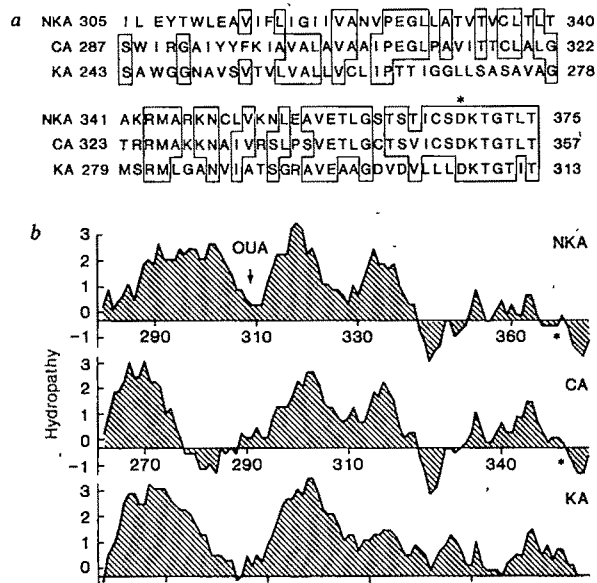


Fig. 4 Energy transduction region and ouabain binding site. **a**, Comparison of the amino-acid sequence of $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ (NKA) with the corresponding sequences of the $\text{Ca}^{2+}\text{-ATPase}$ (CA) and the $\text{K}^+\text{-ATPase}$ B protein (KA) of *E. coli*¹⁸. The sequences compared extend from Ile 305 of the $(\text{Na}^+ + \text{K}^+)\text{ATPase}$, which is on the N-terminal side of H4, to Thr 375 which is six residues to the C-terminal side of Asp 369, the phosphorylation site (indicated by the asterisk). Identical residues are boxed. **b**, Comparison of the hydropathy plots for the $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ (top panel) extending from Glu 280 to Thr 375, which includes H3 and H4 and the phosphorylation site, indicated by the asterisk, with those of the corresponding region for the $\text{Ca}^{2+}\text{-ATPase}$ (middle panel) and the $\text{K}^+\text{-ATPase}$ B protein of *E. coli* (bottom panel). The probable location of the ouabain binding site is indicated. The hydropathy plots are not condensed as in Fig. 2b; nine residue averages for each amino acid have been plotted³¹.

that maps to this region contains membrane-embedded sequences and includes both the phosphorylation site and a site which reacts with a ouabain photoaffinity derivative³⁸. The accessibility of trypsin and chymotrypsin sites at the N-terminal boundary of this peptide, and a trypsin site at the C-terminal boundary, are dependent on the conformation of the enzyme (reviewed in ref. 33). Possible locations of these proteolytic sites are, respectively: Arg 262, which would yield the expected N-terminal isoleucine²⁷; Phe 284, the first residue of H3; and Arg 423, which would yield the expected N-terminal Ala²⁷.

A region located between the trypsin sites was compared with similar sequences of the Ca^{2+} -ATPase and the K^{+} -ATPase B protein of *Escherichia coli*¹⁸. When the full hydropathic plot for the K^{+} -ATPase B protein was compared with that of the $(\text{Na}^{+} + \text{K}^{+})$ -ATPase (data not shown), the only major hydrophobic domains which seemed to be analogous were those next to the phosphorylation site. Furthermore, there was a region of amino-acid homology around the phosphorylation site that extended into H4. This is of interest because H4 is the only major hydrophobic domain that exhibits strong amino-acid homology between the $(\text{Na}^{+} + \text{K}^{+})$ -ATPase and the Ca^{2+} -ATPase, and because of its proximity to the phosphorylation site it would be a likely pathway for transmitting conformational changes into the intramembrane regions of the protein. The amino-acid homology for this section of all three enzymes is shown in Fig. 4a and the hydropathy plots for sequences including H3 and extending through the phosphorylation site are shown in Fig. 4b. The similarity in their hydropathy plots and the amino-acid homology between these eukaryotic and prokaryotic ATPases suggests that the sequence extending from the phosphorylation site into H4 may be an energy transduction pathway⁴¹ into the transmembrane domains involved in cation transport.

Cardiac glycoside binding site

An attractive possibility for the location of the ouabain binding site is the junction between H3 and H4. Studies with anthroyl-ouabain indicate that the binding site is relatively hydrophobic, shielded from water and is close to a Trp residue⁴². This observation prompted the development of a ouabain photoaffinity derivative which is activated by energy transfer from an excited Trp residue; this derivative was incorporated exclusively into the N-terminal half of the protein⁴³. Similar results were obtained with an alkylating-ouabain derivative⁴⁴. The ouabain binding site in this portion of the molecule seems to be localized to the H3-H4 region, but binding has also been demonstrated in the C-terminal region³⁸. It has been suggested that this additional site, which could be at the H5-H6 junction or the H7-H8 junction, is not specific for the steroid-like portion

of the molecule⁴³.

If the path of the polypeptide chain through the membrane is as suggested here, then there are just four short extracellular sequences. Only the H3-H4 junction, which includes residues 307-312 and probably some adjacent residues, seems to have all the properties predicted for the ouabain binding site. First, it is relatively hydrophobic yet should not be embedded in the membrane as it contains two glutamate residues, a Trp and a Tyr. Trp and Tyr, although hydrophobic in some respects, are infrequently buried⁴⁵. Second, the presence of a Trp is expected based on the studies described above and Trp 310 is the only such residue found in the probable extracellular regions of the protein. Third, this site is not homologous with the corresponding region of the Ca^{2+} -ATPase, which does not interact with cardiac glycosides. Fourth, given the inhibitory effects of cardiac glycosides on enzyme activity⁷, this site is ideally situated with respect to the proposed energy transduction region and phosphorylation site.

Conclusions

The probable structure of the catalytic subunit of the $(\text{Na}^{+} + \text{K}^{+})$ -ATPase, deduced from the present work and the studies discussed above, is as follows. The protein consists of 1,016 amino acids and has eight transmembrane domains. Much of the protein, including the hydrophilic N-terminal domain, the sequences between H2 and H3 and those between H4 and H5, is located on the cytoplasmic side of the membrane. The C-terminus and the region between H6 and H7 are also likely to be cytoplasmic. Very little of the protein is extracellular.

As well as the phosphorylation site and the ATP-binding site, several possible functional domains have been identified. The hydrophilic N-terminal domain might function on the cytoplasmic side as an ion-selective barrier which controls access to cation-binding sites. Hydrophobic domains H1 and H2 may form part of a cation channel and the hydrophilic junction between them is a likely candidate for extracellular cation-binding sites. A relatively hydrophobic extracellular cleft between H3 and H4 has the characteristics expected for the digitalis binding site and is connected to the phosphorylation site by a sequence which may be an important component of the energy transduction system.

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Amino-acid sequence of a $\text{Ca}^{2+} + \text{Mg}^{2+}$ -dependent ATPase from rabbit muscle sarcoplasmic reticulum, deduced from its complementary DNA sequence

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We have cloned and sequenced complementary DNA encoding a Ca^{2+} -ATPase of rabbit muscle sarcoplasmic reticulum. We propose a model of the protein which has 3 cytoplasmic domains joined to a set of 10 transmembrane helices by a narrow, penta-helical stalk. In this model, ATP bound to one cytoplasmic domain would phosphorylate an aspartate in an adjoining cytoplasmic domain, inducing translocation of Ca^{2+} from binding sites on the stalk.

THE $\text{Ca}^{2+} + \text{Mg}^{2+}$ -dependent ATPase (Ca^{2+} -ATPase) of rabbit skeletal muscle sarcoplasmic reticulum, first isolated in 1970 (ref. 1), is a mammalian ion-transport enzyme which has been extensively investigated at the molecular level². The membrane-bound enzyme is cleaved instantly by trypsin (at site T_1) into two fragments of equal length, A and B; fragment A is cleaved more slowly (at site T_2) into unequal subfragments A_2 and A_1 (ref. 3). Functional sites have been associated with these fragments, each of which is independently anchored in the membrane². More detailed information on the topography of the enzyme and on the localization of active sites has been obtained from the amino-acid sequence of five cytoplasmic segments of the enzyme which include the amino-terminus (sequence 1), the

carboxy-terminus (sequence 5) and three long, internal segments (sequences 2–4)⁴. Hydrophobic transmembrane sequences have not been obtained, however, leaving a gap in our understanding of the structures through which Ca^{2+} ions are bound and transported across the membrane.

The need to obtain the full sequence of the Ca^{2+} transport enzyme, together with the desirability of obtaining new information about genes for homologous proteins and the expression of these genes during development, has led us to clone complementary DNA encoding two forms of the Ca^{2+} -ATPase. The characterization of one of these cDNA clones has permitted us to determine its complete primary structure and to predict its secondary and tertiary structure.

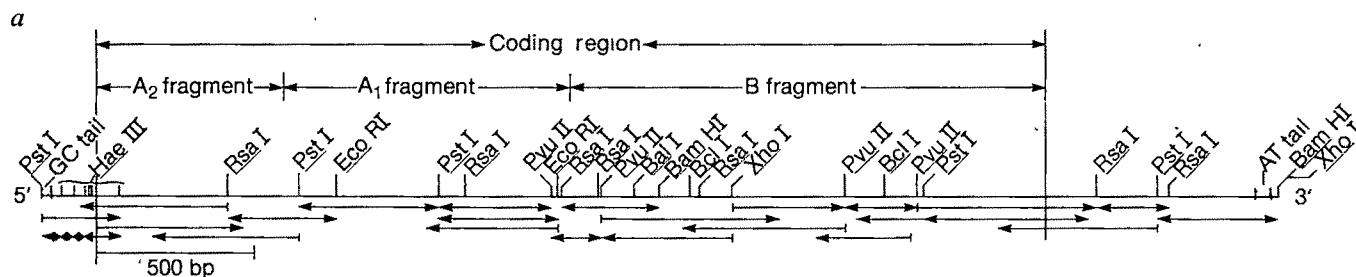


Fig. 1a. Sequencing strategy for cloned cDNA encoding a Ca^{2+} -ATPase protein of rabbit muscle sarcoplasmic reticulum. The restriction map displays six-base restriction endonuclease sites and those four-base restriction sites that were used in sequencing. The coding region is divided into three segments, A_2 , A_1 and B, which correspond to the A and B fragments generated by brief tryptic digestion of the fast twitch skeletal ATPase protein in intact sarcoplasmic reticulum³. **b.** Nucleotide and deduced amino-acid sequence of the Ca^{2+} -ATPase. The amino-acid sequence of the clone bears 84% identity with the fast skeletal ATPase⁴ and identity with a sequence of 17 amino-acid residues ((R)TSMKMFVKGAPEGVIDR beginning at residue 505) found in the Ca^{2+} -ATPase from canine cardiac sarcoplasmic reticulum (Briggs, F. N. *et al.*, in preparation). Nucleotide residues are numbered positively in the 5' to 3' orientation beginning with residue 1 of the ATG codon for the initiator methionine and ending with the last residue before the poly(dA) tail. They are numbered negatively beginning from the 5' side of residue 1 to the beginning of the poly(dG) tail (added to the 5' end of the cDNA before its incorporation into the vector pcD-X³⁶). The deduced amino-acid sequence is shown below the nucleotide sequence. Amino-acid residues are numbered beginning with the initiator methionine below the residues on the left-hand of each row. Polar segments, of sufficient length to be transmembrane, are underlined. Extensions of transmembrane segments and potential transmembrane segments (see Fig. 2) are underlined with a broken line. The major tryptic sites (T_2 and T_1), the sites of phosphorylation (P) and the site of FITC binding (F) are indicated beneath the appropriate amino acids.

Methods. Total RNA was extracted from skeletal muscle of 1–3-day-old rabbits as described previously³⁷, with the following modifications. SDS and diethyl pyrocarbonate were absent from all solutions and RNA was extracted with CHCl_3 /isoamyl alcohol (4:1) before a single step of chromatography on an oligo(dT)-Sepharose column. A series of cDNA libraries was constructed in the vector pBR322 by standard procedures involving first-strand synthesis by reverse transcription of oligo(dT)-primed mRNA, second-strand synthesis with the Klenow fragment of DNA polymerase, removal of the hairpin loop with S_1 nuclease, tailing with deoxycytidine and ligation of the double-stranded cDNA with Pst I-cleaved, oligo(dG)-tailed pBR322. The first library of 3,500 clones was probed with a group of four synthetic oligomeric deoxynucleotides of composition 5'-GC^ATACAT^GAACCA-3 based on the amino-acid sequence (W)FMYA recently determined for a hydrophobic peptide in the B fragment of the Ca^{2+} -ATPase³⁸. This screening yielded the first fast skeletal muscle ATPase clone, pFA1, containing 75 bp of coding sequence lying within a 234-bp Pvu II/ Pvu II fragment near the 3' end of the fast skeletal muscle ATPase clone (the homologous Pvu II/ Pvu II fragment is present in the clone described in Fig. 1). A fragment of 71 bp was cut from pFA1 with Sau 3a and Hae III and used to probe a second library of 50,000 clones yielding six clones of fast skeletal ATPase and two cross-hybridizing clones, pCA1 (1,900 bp) and pCA2, which contained all of the residues in pCA1 but terminated earlier in the 3'-untranslated region. These clones were sequenced and the 234-bp Pvu II/ Pvu II fragments from both groups of clones were used to probe a library of 1.6×10^6 clones in the vector pcD-X using the method of Okayama and Berg³⁹. This library yielded 3 fast skeletal muscle clones and 16 cross-hybridizing clones. The longest cross-hybridizing clone, pCA3 (3,722 bp), was restriction-mapped as illustrated above and clones pCA3 and pCA4 (3,602 bp) were used for sequence analysis. Restriction endonuclease fragments were subcloned into the vectors pEMBL8 and pEMBL9 to obtain sequences in both orientations³⁹. Sequences initiated at sites other than restriction sites were obtained from the shorter clones that terminated in regions inaccessible by suitable restriction endonuclease fragmentation. Production of single-stranded DNA suitable for DNA sequencing was induced by superinfection with the phage IR1 (ref. 20). Single-stranded DNA was sequenced by the dideoxy method of Sanger *et al.*⁴⁰

initiator Met codons in the first 128 nucleotides upstream of the ATG in our ATPase transcript suggests that this region is untranslated, consistent with our earlier observations¹¹ that the fast skeletal muscle Ca^{2+} -ATPase is synthesized without an amino-terminal signal sequence.

We do not yet know whether the 128 nucleotides upstream of the first Met codon represent all, or only part, of the 5'-untranslated region of the ATPase transcript. Northern blot analysis of neonatal rabbit muscle messenger RNAs with our cDNA probes showed a hybridizable transcript of ~3.7 kilobases, suggesting that the cDNA clones are very close to full length. The 5'-untranslated sequence contained 65 G, 44 C, 16 A and 3 T residues. Thus, the nucleotide content of this sequence resembled the 5'-untranslated region of the $(\text{Na}^+ + \text{K}^+)$ ATPase gene transcript published elsewhere in this issue¹², but was even more G+C-rich (85%).

The 3'-untranslated region terminated in a poly(dA) tail. The sequence upstream of the tail did not contain a perfect AATAAA polyadenylation signal¹³, but the sequence AATACAA terminated 21 bases before the poly(dA) tail and was followed closely by the sequence TGTGATGC, which is very similar to the consensus sequence YGTGTTY found between many polyadenylation signals and their poly(A) tails¹⁴.

A sequence beginning 51 bases downstream of the stop codon (nucleotides 3,045–3,074) contained an interesting homology with the core sequence of the murine IgG enhancer sequence, although loop-outs would be necessary in order to achieve maximal homology. Our sequence GTGCGTTTTAGCAACACACCTGCCAGCCC (nucleotides 3,045–3,074) resembled the sequence GTGGTTTGAACACTCTGTCAGCCC reported by Hayday *et al.*¹⁵ as comprising part of an enhancer sequence.

Homology between ATPases

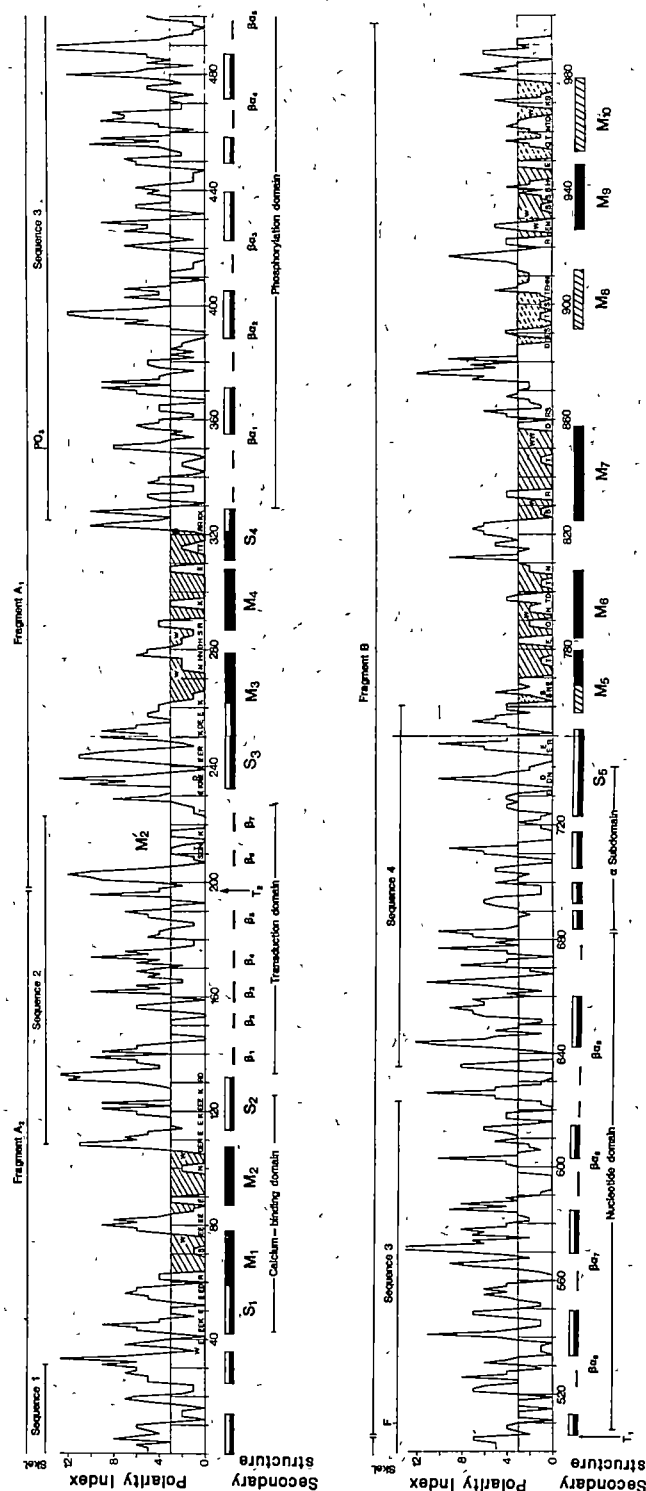
Homology between the amino-acid sequences of the extramembranous segments of the Ca^{2+} -ATPase and the K^+ -ATPase of *Escherichia coli* has already been pointed out¹⁶. There was ~50% homology in some limited regions covering a total of ~180 residues. Note that there is little or no homology in the

transmembrane regions beyond a general hydrophobicity, but that the location of these hydrophobic sequences in the overall sequence is very similar in the two ATPases. The regions of homology with the $(\text{Na}^+ + \text{K}^+)$ ATPase¹² were more extensive and included the transmembrane segments M_4 , M_6 and M_7 (see Fig. 2). The first transmembrane segment, M_1 , is interesting in that both Ca^{2+} and $(\text{Na}^+ + \text{K}^+)$ ATPase sequences showed separate homologies with the M_1 section of the K^+ -ATPase while showing little resemblance to each other.

Secondary and tertiary structure

In the absence of a database of membrane proteins of known structure, empirical prediction of their secondary structure is unreliable¹⁷ as strongly hydrophobic segments are almost invariably predicted as β -strands. Therefore, we have identified the

Fig. 2 Polarity profile and secondary structure analysis of the Ca^{2+} -ATPase aligned with the tryptic fragments and the previously determined major sequences of the fast skeletal muscle ATPase. The top line ('fragment') represents tryptic fragments (A_2 , A_1 and B) of the fast skeletal muscle ATPase⁴. The second line ('sequence') indicates previously determined major sequences of the fast skeletal muscle ATPase⁴, showing phosphorylation and fluorescein-reactive sites. The polarity index of amino-acid triplets is shown below these two lines (only polar residues score: S, T=1; N, Q, H=2; D, E, K=3; R=4). The plot is designed to emphasize polar interruptions of potential transmembrane helices. In a small helical bundle any polar interruption of >2 residues is likely to force at least one polar residue out into the hydrophobic lipid phase. For this reason, penalty points are added to the triplet score equal to the lowest score of the two outer residues. Any run of 21 residues rising nowhere above polarity 3 is a putative transmembrane sequence. Individual polar residues within putative transmembrane stretches are shown, facilitating evaluation of their influence. Other individually noted residues are charged residues in the α -helices that link the globular domains to the membrane, and tryptophans (changes in tryptophan fluorescence have been used to monitor conformational changes in the reaction cycle). We note that 11 out of 13 residues are located in transmembrane segments). Secondary structure is identified by the template fitting procedure of Taylor and Thornton¹⁹ and applied to long extramembranous segments: $\blacksquare$, Amphipathic helix; --- , β -strand; --- , hydrophobic transmembrane helix. The nomenclature for structural domains is shown at the bottom of the figure. The transmembrane domain consists of hydrophobic helices M_1 – M_{10} ; the stalk of amphipathic connecting helices S_1 – S_5 ; the transduction domain of residues 133–238 (antiparallel β -sheet); the phosphorylation domain of residues 330–505 (parallel β -sheet); the nucleotide-binding domain of residues 506–680 (parallel β -sheet); and the α -helical sub-domain of residues 681–740.



transmembrane sequences by their hydrophobicity and applied standard methods only to the extramembraneous regions. These regions of the ATPase are mainly on the cytoplasmic side of the membrane⁴ and are separated by transmembrane hydrophobic hairpins, which we have located by the polarity plot shown in Fig. 2. This plot gives results similar to those provided by the method of Kyte and Doolittle¹⁸ but has the advantage of indicating explicitly the nature and location of polar interruptions. Combination of this information with an α -helical net facilitated analytical evaluation of potential transmembrane segments.

An earlier analysis of secondary structure⁴ detected several regions of predominant β -sheet and α -helix and one section of α/β alternation which was tentatively identified as a nucleotide-binding site⁹. Clear evaluation was prevented by the uncertain priorities when α and β were predicted simultaneously, and by the incomplete sequence. Therefore, we used the improved procedure of Taylor and Thornton¹⁹ which starts from a prediction of α -helices, β -strands and β -turns and resolves some of the ambiguities in priority of strand and helix by comparing patterns of hydrophobicity and of supersecondary structure with known structures. Our general conclusions are shown in Fig. 2 and discussed below.

Various experiments have enabled functional sites to be assigned to the three tryptic fragments of the ATPase and their constituent sequences. Fragment A₂ has been implicated in Ca²⁺ binding and translocation²⁰⁻²³. The phosphorylation site²⁴ is near the amino-terminus of sequence 3 (see Fig. 2) and the nucleotide-binding site⁹ near the amino-terminus of fragment B. We have used these three sites as a basis for naming functional domains of the tertiary structure (Figs 2, 4). We have distinguished a fourth domain, the transduction domain, based on evidence that cleavage at the second tryptic site, T₂, prevents Ca²⁺ transport without inactivation of the ATPase²⁵. A similar site has been observed in the (Na + K)⁺ATPase²⁶.

Hydrophobic segments

The first pair of transmembrane segments (M₁ and M₂) are clearly defined by the high density of charged residues on either side of the hydrophobic segments. The next hydrophobic segment, M₂' (207-238), contains a typical β -bend sequence, SGTN, and, for this and other reasons (see below), it is unlikely to be transmembrane helix. A second pair of transmembrane segments (M₃ and M₄) is again clearly defined. From this point, at the beginning of the longest extramembraneous segment, there are no more nonpolar interruptions until residue 770. In earlier folding models of the ATPase^{2,4}, a transmembrane loop was arbitrarily introduced between amino-acid sequences 3 (residues 325-622) and 4 (637-759). We show here that this was unwarranted as these sequences are separated by only 15 relatively hydrophilic amino acids. These combined sequences create a polar cytoplasmic segment 450 residues long. The segment from residue 770 to the carboxy-terminus is largely hydrophobic and is broken only by three polar intrusions, each of ~20 residues. The first of the carboxy-terminal hydrophobic segments is long enough to pass twice across the membrane, creating transmembrane segments M₅ and M₆. There are four more possible transmembrane segments (M₇, M₈, M₉ and M₁₀), but two of these are either short or contain several polar residues (Fig. 2; hatched sections). There is a maximum of six crossings of the membrane in this carboxy-terminal region, but there may be as few as four. Our assignments predict that the polar connections on the luminal face, except for the connection between M₇ and M₈, will be short.

Extramembraneous domains

Before the first membrane hairpin (residues 1-59) there are three α -helices, the third of which (S₁) contains five aligned Glu residues and runs straight from the cytoplasm into the membrane (M₁) without any obvious bend (Fig. 3). The next cytoplasmic domain (residues 109-262) contains the second tryptic site (T₂). The central region (122-230) contains seven β -strands, seven

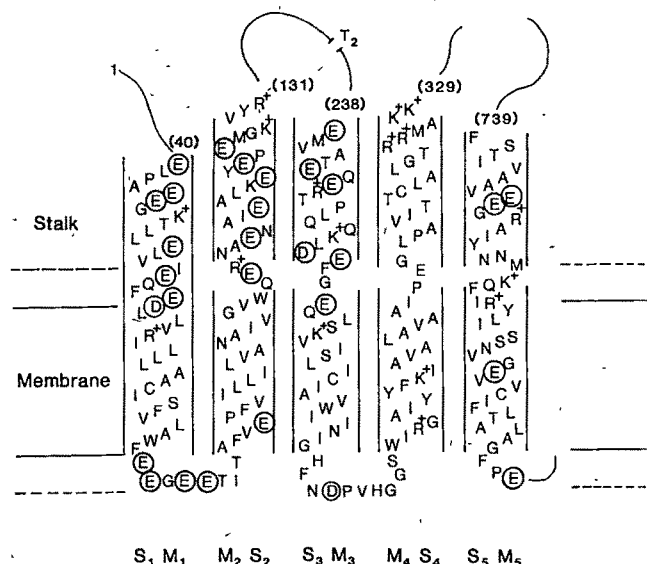


Fig. 3 Structure of the connecting stalk and attached transmembrane helices. This includes the probable Ca²⁺-binding domain region of the ATPase and part of the transmembrane channel. Note that the first three helices of the stalk are markedly amphipathic, with one highly charged face. There are 9 charges (5+, 4-) in this section of the transmembrane region, which increases to 21 (7+ and 14-) if the polar head-group regions are included. It is likely that the two high-affinity sites for Ca²⁺ are on the cytoplasmic face near the polar head groups, whereas the low-affinity sites involve a distinct set of Glu residues in the luminal head-group region.

ated by short loops, so that it is likely to fold to give an antiparallel β -sandwich²⁷. It is connected to the membrane by amino- and carboxy-terminal α -helices (S₂ and S₃), each of ~20 residues. If the segment M₂ were transmembranous it would deplete the domain of two β -strands, which would diminish its structural plausibility. It would also invert the topology of succeeding loops unless a further transmembrane segment were postulated (for example, by including the hydrophobic section of S₄). The homologous region of the (Na⁺ + K⁺) ATPase is more polar and seems not to have transmembrane sequence at this point¹². Therefore, we conclude that M₂' is part of the cytoplasmic domain.

The long cytoplasmic segment (residues 308-758) contains both the phosphorylation site and the nucleotide-binding site, separated by the tryptic cleavage site T₁. Most of this region (337-680) is predicted to consist of alternating β -strands and α -helices, which would fold to give parallel β -sheets²⁷. Both its size and the central tryptic cleavage site suggest that this region folds into two separate domains, the first of which is phosphorylated by a molecule of ATP bound to the second. In this respect, as well as in secondary structure, these domains resemble those of phosphoglycerate kinase and hexokinase²⁸. There is a further resemblance in that the second domain is followed by a short (60-residue) section of α -helices. In these proteins and in alcohol dehydrogenase such a helical addendum rejoins the first domain, giving a second interdomain link and establishing the hinge between the domains²⁸. These two parallel β -domains are typical of their class in that most of the β -strands are hydrophobic on both faces; this is particularly marked in the nucleotide domain. However, they lack the amino-terminal GXGXXG sequence characteristic of the nucleotide domain of many dehydrogenases and protein kinases²⁹.

Stalk region

The segments that connect the globular domains to the membrane are of particular interest as they are likely to include the Ca²⁺-binding region and to transmit movements from the phosphorylation and nucleotide domains that bring about ion translocation. There are five of these connecting segments if we

include the amino-terminus as a small additional domain. Because the connecting segments will form the narrow neck of the ATPase molecule, which can be seen in electron micrographs of negatively stained vesicles³⁰, we refer to them as stalk segments (S_1 – S_5). All but S_4 are strongly predicted to be helices of length ~ 3 nm. When the residues are written on an α -helical net (Fig. 4) the helices are clearly amphipathic, having a high density of charged residues on one face. The highly conserved segment S_4 , which connects M_4 to the phosphorylation site, is different in having a hydrophobic amino-terminus which gives priority to β -strand over α -helix. Nevertheless, a helix is likely to be favoured because of close association of the section with the other four helices of the stalk. Helices S_1 , S_2 and S_3 are notable for their 16 aligned Glu residues (Fig. 3) which must contribute to the Ca^{2+} -binding sites. With one aspartate, they are the only negatively charged groups adjoining the membrane in the A_2 fragment. There is no sequence there or elsewhere in the molecule which resembles the DXDXDX loop characteristic of the EF hand of Ca^{2+} regulatory proteins³¹. Five or six of these Glu residues will be in the lipid polar head group region on the cytoplasmic surface of the membrane, and four of these will be in the bend regions between the S and M helices (Fig. 3). On the luminal side of the M_1 – M_2 loop there is another cluster of four Glu residues (EEGEE), which is a strong candidate for the low-affinity sites from which Ca^{2+} is released into the lumen. We suggest that the low-affinity Ca^{2+} -binding sites on the luminal face are constituted from a completely different set of residues than the high-affinity sites.

Topography of ATPase domains

An outline of the structure of the Ca^{2+} -ATPase can be deduced from electron micrographs showing a globule attached to the membrane by a narrow stalk³⁰. The transmembrane helices are observed on freeze fracture as intramembranous particles³². In our planar diagram (Fig. 4) we suggest that the three extramembranous globular domains form a headpiece which lies on top of a stalk comprised of five helices which are, in turn, adjoined to five transmembrane helices. These transmembrane helices together with five additional helices at the carboxy-terminal end of the molecule, form the intramembranous basepiece.

Our knowledge of domain function, derived from extensive kinetic and labelling studies³³, suggests that all three extramembranous domains are highly interactive. The nucleotide domain is relatively independent of the rest of the molecule in that it can be blocked by FITC without preventing either phosphorylation from inorganic phosphate or Ca^{2+} transport energized by acetyl phosphate³⁴. Nevertheless, it is probably folded beside the other two globular domains, as it has to transfer the γ -phosphate of ATP to the phosphorylation site on the phosphorylation domain. There is also some interaction of this domain

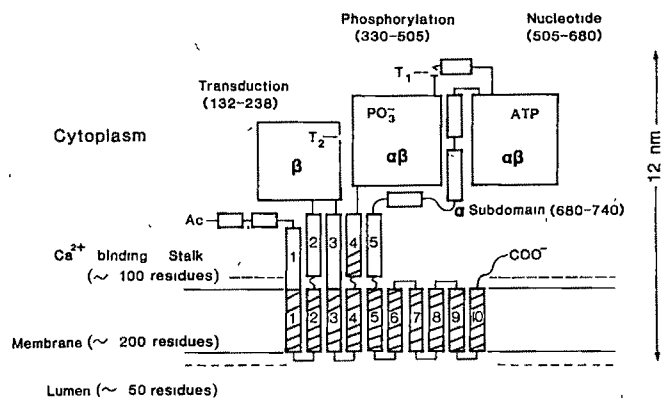


Fig. 4 Assembly of ATPase domains. The predicted arrays of helices and the three major domains are laid out in a planar diagram. In the ATPase molecule the helices would form tight clusters. The ATP-binding domain would be folded beside the other two major domains, permitting interaction among all three and accounting for the approximately triangular molecular profile derived from electron micrographs of ordered arrays by optical diffraction⁴¹ and for the molecular shape deduced from hydrodynamic measurements³⁵ and electron microscopy³⁰.

with the Ca^{2+} -binding site as, for example, bound ATP accelerates the binding of Ca^{2+} , as well as influencing other stages of the reaction cycle³³. The planar diagram, while speculative, represents an advanced model for the secondary and tertiary structure of the Ca^{2+} -ATPase on which refinements and prediction of mechanism may eventually be based.

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Human transforming growth factor- β complementary DNA sequence and expression in normal and transformed cells

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The partial amino-acid sequence of purified human transforming growth factor- β (TGF- β) was used to identify a series of cDNA clones encoding the protein. The cDNA sequence indicates that the 112-amino acid monomeric form of the natural TGF- β homodimer is derived proteolytically from a much longer precursor polypeptide which may be secreted. TGF- β messenger RNA is synthesized in various normal and transformed cells.

Two different peptides which can induce the reversible phenotypic transformation of mammalian cells in culture are termed transforming growth factor (TGF) α and β . TGF- α is synthesized by various transformed cell lines^{2,3} and competes with epidermal growth factor (EGF) for binding to the same cell-surface receptor². A 50-amino-acid-long TGF- α species has been purified and shown to share sequence homology with EGF⁴. This TGF- α is initially synthesized as part of a 160-amino-acid-long precursor molecule which undergoes NH₂- and C-terminal proteolytic processing to yield the mature peptide^{5,6}.

TGF- β has been isolated from tumour and normal cells and tissues⁷, including kidney⁸, placenta⁹ and blood platelets^{10,11}. The relatively high levels of TGF- β present in platelets, which also contain platelet-derived growth factor (PDGF), have led to the suggestion that TGF- β plays a role in wound healing¹². It is a homodimer of relative molecular mass (M_r) ~25,000 (ref. 11) which recognizes a cell surface receptor distinct from the EGF receptor^{13,14}. Treatment of NRK fibroblasts with TGF- β does, however, result in an increase in the number of membrane receptors for EGF¹⁵, which may explain the ability of TGF- β to greatly potentiate transforming activity of EGF and TGF- α on these cells⁷. Moreover, TGF- β alone can induce AKR-2B fibroblasts to form colonies in soft agar¹⁶. In addition to stimulating cell proliferation, TGF- β has recently been demonstrated to inhibit the anchorage-dependent growth of a variety of human cancer cell lines¹⁷. TGF- β may be identical or very similar to a growth inhibitor isolated from African green monkey (BSC-1) cells¹⁸ and has many properties in common with the tumour inhibitory factor TIF-1 (ref. 19). Whether TGF- β acts to stimulate or inhibit the growth of a particular cell type seems to depend on many variables, such as the physiological condition of the cell and the presence of additional growth factors.

Here we report the amino-acid sequence of human TGF- β determined from direct protein sequencing and cDNA cloning. The nucleotide sequence of the TGF- β cDNA reveals that the 112-amino-acid-long TGF- β monomer is initially synthesized as part of a precursor polypeptide. Northern hybridization analysis demonstrates that the TGF- β mRNA is present in normal cells and in many types of tumour cells.

Purification and sequence analysis

A purification scheme has been developed by Assoian *et al.*¹¹ which yields TGF- β from human blood platelets, a major site of TGF- β storage. This procedure was modified and used to obtain human TGF- β with a purity of >95% for subsequent sequence analysis (Fig. 1 legend). In agreement with previous work¹¹ the non-reduced TGF- β migrated as an M_r 25,000 protein in a SDS polyacrylamide gel, whereas reduction with β -mercaptoethanol converted it into an M_r 12,500 species. This

chains linked by intermolecular disulphide bridges. The purified TGF- β was reduced, alkylated and subjected to amino-terminal sequence analysis. The amino-acid sequences of several peptides obtained after clostripain digestion of TGF- β were also determined. These analyses yielded a 60-residue long contiguous sequence starting from the amino terminus (Fig. 1a) as well as several shorter peptide sequences (data not shown). The NH₂-terminus of the human TGF- β is identical to the previously determined NH₂-terminal sequence of bovine TGF- β ⁸. The fact that no heterogeneity was seen during the NH₂-terminal sequence analysis, and that all TGF- β sequences obtained could be accounted for by an M_r 12,500 polypeptide, provide additional evidence for the homodimeric nature of this protein.

Isolation of TGF- β cDNAs

The approach we followed for the identification of the nucleotide sequence encoding TGF- β was similar to the strategy used previously for TGF- α ³. Long oligonucleotides designed on the basis of the partial protein sequence were used as hybridization probes for the identification of a TGF- β exon in a human genomic DNA library, which was then used as a probe for the isolation of TGF- β cDNAs.

Two 44-mer deoxyoligonucleotides, β LP1 and β LP2, complementary to sequences encoding amino acids 3-17 and 30-44, respectively, were chemically synthesized (Fig. 1a). The choice of these nucleotide sequences was based on previously described considerations⁵. In addition, 16 14-mers were synthesized which are complementary to all possible codons for amino acids 13-16 (Fig. 1a). A human genomic DNA library²⁰ was screened under low stringency hybridization conditions using ³²P-labelled β LP1 as probe. One of the 58 hybridizing phage, β λ58, also hybridized with the 44-mer β LP2 and with the pool of 14-mers. Nucleotide sequence analysis of this clone revealed the presence of a TGF- β exon encoding amino acids 10-60 of TGF- β . Only about half of the sequence corresponding to the β BP1 oligonucleotide is present. Nevertheless, the probe hybridized because of the correct assignment of 23 of the 24 nucleotides in the portion of the probe corresponding to the exon sequence (data not shown).

To obtain the entire TGF- β coding sequence, this exon was used as a probe to screen a λ gt10-based cDNA library derived from human term placenta mRNA. The screening of ~750,000 oligo(dT) primed cDNA clones resulted in the isolation of one TGF- β cDNA (λ BC1) of ~1,050 base pairs (bp). The previously determined partial TGF- β sequence established the reading frame and revealed the sequence encoding the complete TGF- β polypeptide, followed by a stop codon 20 bp from the 3' end of the cDNA.

Northern hybridization^{21,22} using the λ BC1 cDNA insert as probe showed that TGF- β mRNA of ~2.5 kilobases (kb) is

lines. Extensive screening of cDNA libraries prepared from both these cell lines and of an additional placenta cDNA library yielded many different TGF- β cDNAs. Most of these seemed to contain sequence inversions generated during cDNA preparation ('Belgian' clones²³). In addition, single base deletions and substitutions were found in some of the cDNAs. Because of the high incidence of cDNA artifacts, presumably resulting from specific features in the mRNA or cDNA structure, all parts of the sequence were confirmed by sequencing at least two different cDNAs. Alignment of different cDNA sequences (Fig. 1b) enabled us to establish 2 kb of sequence preceding the TGF- β termination codon (Fig. 1c).

None of the 70 TGF- β cDNAs isolated from different oligo(dT)-primed cDNA libraries contained more than a few nucleotides of 3' untranslated sequences. As introns are rarely found in 3' noncoding regions, the 3' untranslated sequence was determined using cloned genomic DNA. DNA sequence analysis of phage β 58 revealed the presence of an exon encoding the carboxy-terminal part of TGF- β , followed by the stop codon and the 3' untranslated region (Fig. 1c). An AATAAA hexanucleotide sequence²⁴ was encountered 500 bp downstream from the termination codon, thus permitting an assignment of the putative polyadenylation site. The calculated size of TGF- β mRNA is in close agreement with the 2.5 kb length determined from the Northern hybridization experiments (Fig. 2).

Carboxy-terminus of TGF- β

The cDNA sequence establishes that TGF- β is synthesized as the C-terminal segment of a precursor polypeptide. As several proteins undergo post-translational removal of C-terminal sequences, we determined the actual C-terminal amino-acid sequence of TGF- β . The cDNA sequence predicted the presence of a single methionine near the C-terminus in TGF- β . The protein was reduced, alkylated and cleaved with cyanogen bromide (CNBr). Sequence analysis of the mixture of peptides resulting from the CNBr digestion revealed the C-terminal sequence predicted from the cDNA (data not shown), although it cannot be excluded that a fraction of the molecule had undergone C-terminal proteolysis.

Unmodified TGF- β was also treated with CNBr. Cleavage at the methionine residue resulted in the complete loss of biological activity, showing that at least part of this C-terminal octapeptide is needed for biological activity (data not shown).

mRNA in different cell types

The availability of a TGF- β cDNA allowed us to screen various cells for TGF- β mRNA (Fig. 2). TGF- β mRNA was detectable in all human tumour cell lines including Wilms Tumour, A172 (glioblastoma) and the carcinoma cell lines T24 (bladder carcinoma), A431 (Squamous epidermoid carcinoma), MCF-7 (mammary carcinoma) and KB (nasopharyngeal carcinoma). HT1080, a fibrosarcoma-derived cell line, which we had chosen as a source of mRNA for the cDNA cloning, contained relatively high levels of TGF- β mRNA. TGF- β mRNA was not only present in cell lines derived from solid tumours of meso-, endo- and ectoblastic origin, but was also detectable in tumour cell lines of hematopoietic origin, for example Daudi (Burkitt lymphoma B lymphoblast), Raji (Burkitt lymphoma B lymphoblast) and Molt-4 (T-cell leukaemia). The presence of TGF- β mRNA is not restricted to tumour cells, as it is clearly detectable in placenta and peripheral blood lymphocyte (PBL) mRNA. Strikingly, the level of TGF- β mRNA is significantly elevated after mitogenic stimulation of PBLs. TGF- β mRNA was not detectable in human liver, yet was present in the HEP-G2 hepatoma cell line. In all cases, the TGF- β mRNA migrated as a species with an apparent length of ~2.5 kb.

Discussion

Analysis of several overlapping cDNAs and gene fragments has led to the determination of a continuous sequence of 2,439 bp corresponding to the TGF- β precursor mRNA. An initiator ATG is located 842 nucleotides from the 5' end and establishes

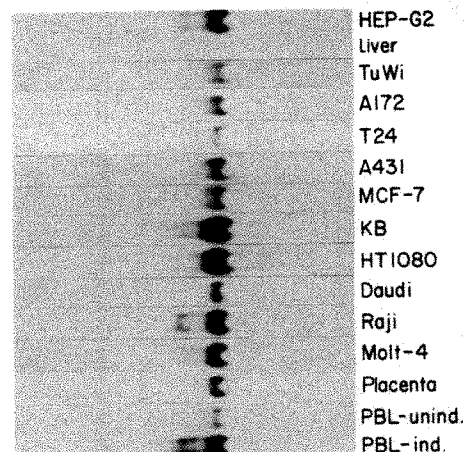


Fig. 2 Northern hybridization of mRNA from different sources with a TGF- β cDNA as probe. The cell source of the polyadenylated RNA is shown above each lane. The following cell lines were used: the hepatoma HEP-G2, Wilms tumour TuWi, glioblastoma A172, bladder carcinoma T24, squamous epidermoid carcinoma A431, mammary carcinoma MCF-7, nasopharyngeal carcinoma KB, fibrosarcoma HT1080, Burkitt lymphoma B lymphoblasts Daudi and Raji and T lymphoblast Molt-4. The cells growing in the monolayer were collected at confluency. The peripheral blood lymphocytes were prepared and mitogen-induced with staphylococcal enterotoxin B and phorbol myristate as described previously⁵⁴. RNA was prepared 24 h after induction. Comparison with the position of the 28S and 18S ribosomal RNA on the gel suggests a length of ~2.5 kb for the TGF- β mRNA. 4 μ g of polyadenylated mRNA was electrophoresed into a formaldehyde 1.2% agarose gel²¹ and blotted onto nitrocellulose filters²². The ³²P-labelled⁵⁶ EcoRI cDNA insert of λ BC1 was used as a probe under high stringency conditions (see Fig. 1 legend).

a coding sequence of 1,173 nucleotides, thus encoding a 391 amino-acid long polypeptide (Fig. 1b, c). Several areas in the cDNA sequence have an exceptionally high G-C content. In particular, the first 450 bp at the 5'-terminus has regions with >80% G-C content. The location of these G-C rich regions coincides with the areas in which the many cDNA cloning artifacts occurred and where partial length cDNAs were obtained. It is likely that the first ATG triplet located at position 842 (Fig. 1c) is the initiation codon as it is preceded in the same reading frame by several stop codons and it could initiate translation into a continuous sequence of a large polypeptide which comprises the TGF- β monomer. However, the sequence context at this ATG is not in good agreement with the proposed initiation codon consensus sequence G/ACCATGG²⁵. Furthermore, this ATG is localized in a very G-C rich area. A second ATG, found in the same reading frame at position 954, is in much better agreement with the initiation codon consensus sequence and is localized within a 40-nucleotide region of relatively low G-C content (~50%). This ATG may be more accessible to the ribosome, especially as it is localized within a large G-C region. Although it is more likely that translation starts at the first ATG, it is conceivable that the second ATG could also be used to initiate translation of a smaller TGF- β precursor.

The 5' untranslated region of the TGF- β mRNA is at least 841 nucleotides long and contains a 61-nucleotide-long sequence (positions 192-252) consisting almost exclusively of purines. The biological relevance of the exceptionally long 5' untranslated region of high G-C content is unknown, but it is similar to the structural organization of the mRNAs for *c-myc*²⁶ and insulin-like growth factor-2 (ref. 27). However, there is no striking sequence homology between these sequences. The first 120 bp of the TGF- β cDNA can theoretically be folded into hairpin loop structures with a calculated stability of -92 kcal (data not shown). It is tempting to suggest that the long 5' untranslated sequence and these hairpin loop structures could play a role in mRNA stability or in the regulation of transcription.

The stop codon at residue 2,016 is immediately followed by a remarkable, G-C-rich sequence of 75 nucleotides (Fig. 1c). This sequence consists of multiple repeats of CCGCC and ends with GGGGGC. The peculiar nature of this sequence is probably responsible for the fact that the 3' untranslated end of the mRNA could not be cloned as a cDNA sequence, perhaps because of the inability of the *Escherichia coli* DNA polymerase I to use this sequence as a template for the second strand cDNA synthesis. Similar repeat sequences have been found in the promoter regions of the genes for human dihydrofolate reductase²⁸, human adenosine deaminase²⁹ and Herpesvirus thymidine kinase³⁰. In the latter case, McKnight *et al.*³⁰ have shown that these structural elements are important in transcription efficiency. In addition, it has been shown that the promoter specific transcriptional factor Sp1 binds to such sequences in the simian virus 40 early promoter region and in a related monkey promoter³¹. In all these cases the G-C rich repeats are followed closely by the Goldberg-Hogness TATA sequence. In the case of TGF- β , however, these sequences are located in the 3' untranslated region of the gene, but, interestingly, are also followed by a TATA-like sequence. The hexanucleotide AATAAA, ~500 nucleotides downstream from the stop codon, probably functions as the TGF- β polyadenylation signal as this would agree with the size of TGF- β mRNA estimated from Northern hybridizations. Benoist *et al.*³² have proposed a consensus sequence TTCCTGCG which immediately precedes the poly(A) tail. A similar sequence, TTCAGGCC, follows the AATAAA sequence in the TGF- β mRNA, providing further support for the assignment of the polyadenylation site at position 2,539 (Fig. 1c).

The TGF- β cDNA encodes a polypeptide of 391 amino acids, assuming that the first ATG is used for translational initiation. Comparison with the experimentally determined NH₂-terminus of TGF- β shows that the C-terminal 112 amino acids constitute the TGF- β sequence. The TGF- β monomer is cleaved from the precursor at the Arg-Arg dipeptide immediately preceding the TGF- β NH₂-terminus. Similar proteolytic cleavage sites have been found in several other polypeptide precursor sequences^{33,34}. Determination of the hydrophobicity profile³⁵ predicts that this Arg-Arg sequence is located within a hydrophilic region which would make it accessible to a trypsin-like protease. Whereas cleavage of the precursor gives rise to the mature TGF- β monomer, it is not known if other biologically active peptides are also released. The TGF- β precursor contains several pairs of basic residues (Fig. 1c) which could also undergo post-translational cleavage and give rise to separate polypeptide entities. However, the TGF- β polypeptide itself contains two Arg-Lys dipeptides which apparently are not cleaved. The TGF- β precursor contains three potential N-glycosylation sites³⁶, none of which are localized within the TGF- β polypeptide (Fig. 1c).

The 112 amino-acid sequence of TGF- β contains nine cysteines, whereas the rest of the precursor contains only three (positions 33, 224 and 226; Fig. 1c). There are no data either on the location of the disulphide bridges or on which cysteines are involved in the interchain disulphide linkage of the TGF- β dimer. Previous studies have shown that reduction of the TGF- β dimer of *M*_r 25,000 generates a single band of *M*_r 12,500 on denaturing polyacrylamide gel¹¹. Sequence analysis of the TGF- β amino-terminus and of the TGF- β peptides obtained after clostripain digestion strongly suggest that the TGF- β dimer consists of two identical polypeptides. This homodimeric nature is also supported by the presence of only a single hybridizing DNA fragment on Southern hybridization of human genomic DNA with a TGF- β exon probe (data not shown). Chou-Fasman analysis of the secondary structure shows³⁷ that the TGF- β polypeptide contains extensive β -sheet with little α -helicity. The region immediately preceding the basic dipeptide cleavage site is probably α -helical.

TGF- β is stored in relatively large quantities in platelets and is also found in association with mammalian cells. It is also released into the culture medium of these cells and, therefore,

is probably a secreted protein. The deduced polypeptide sequence of the 391-amino-acid-long TGF- β precursor shows a contiguous sequence of 16 hydrophobic residues (positions 8-23, Fig. 1c). This sequence may constitute the hydrophobic core³⁸ of an NH₂-terminal signal peptide involved in protein secretion. It is not known at which residue this cleavage by the signal peptidase could occur, but comparison with other known signal peptides shows an average signal peptide length of 20-30 amino acids³⁸. The mature TGF- β would subsequently be released from the precursor as a result of the cleavage by a dibasic peptidase, localized at the external side of the membrane³⁹. Translational initiation at the second ATG (position 954) would result in a 354-amino-acid-long precursor devoid of the putative signal peptide.

Most growth factors seem to be made only by specific cell types. PDGF is present in or released by platelets, endothelial cells, smooth muscle cells and various tumour cells. TGF- α is made by tumour cells of non-haematopoietic origin but has not been demonstrated to be synthesized by any normal adult cells. Previous studies have shown that TGF- β can be found in kidney⁸, placenta⁹ and platelets^{10,11} and is released by several 'normal' and transformed fibroblast cell lines. We examined several types of normal and transformed cells from different embryonic origin for the synthesis of TGF- β mRNA. Our results show that all cell types except adult liver examined contain TGF- β mRNA. The amount of TGF- β mRNA seems to correlate with the degree of mitotic activity, which may explain our inability to detect TGF- β mRNA in adult liver tissue. Furthermore, TGF- β mRNA levels are higher in mitogen-induced lymphocytes than in unstimulated lymphocytes. This correlation also seems to hold for the higher levels of TGF- β expression in transformed cells compared with their non-transformed counterparts⁴⁰.

It is clear that much additional work is needed to elucidate the role of TGF- β during cell division and propagation and in the establishment and maintenance of the malignant phenotype. The knowledge of the structure of TGF- β and its precursor and the use of TGF- β cDNAs as molecular probes will certainly contribute to this understanding.

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LETTERS TO NATURE

Evidence for H α emission associated with the HI bridge connecting the Small and Large Magellanic Clouds

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The bridge of matter connecting the Small Magellanic Cloud (SMC) with the Large Magellanic Cloud (LMC) was first detected thanks to radio observations, and detailed maps of this HI bridge are now available¹. Observations of the Magellanic system at H α wavelength reported by Johnson *et al.*² in 1982 seem to show a diffuse H α emission associated with the HI bridge. As they suggested, it is necessary to confirm this association by obtaining H α profiles in the direction of this bridge. We did this in February 1984 with the help of a scanning Perot-Fabry interferometer and a photon-counting system at the 1.5-m European Space Observatory (ESO) telescope. The choice of the area to be observed was based on recent observations³ with the Very Wide Field Camera (VWFC) on board Spacelab 1 (November 1983) showing an extension of the SMC towards the LMC at wavelengths of 1,930 and 1,650 Å. Detection of H α emission would check that the ultraviolet emission was emanating from the bridge connecting the two galaxies by showing that the cloud of hot stars giving rise to this ultraviolet emission was ionizing part of the HI bridge.

The patchy diffuse area detected by Courtès *et al.* with the VWFC³ is $\sim 1^\circ \times 2^\circ$ in size. Courtès suggested that we should obtain profiles of the H α emission line where ultraviolet maxima had been found ($\alpha = 1$ h 50 min, $\delta = -74^\circ 17'$ and $\alpha = 2$ h 10 min, $\delta = -74^\circ 34'$).

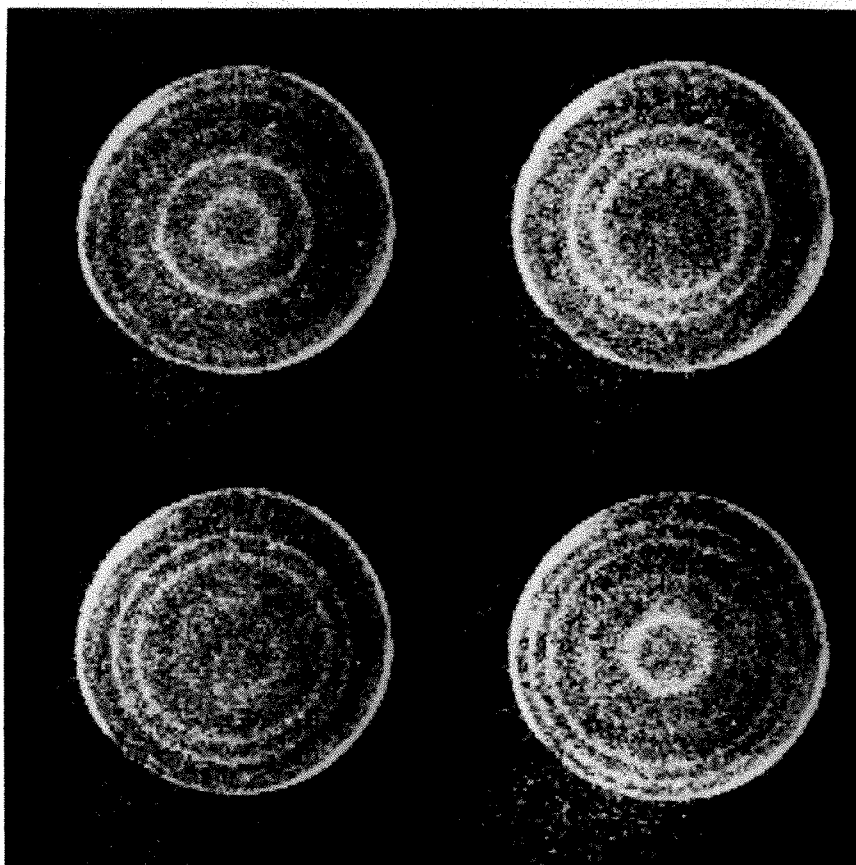
The instrument used for our observations has been described elsewhere⁴. We used a simpler version of the CIGALE experiment at the 1.5-m ESO telescope. The focal reducer was a smaller one, which was operated manually and which reduced the f :15 aperture ratio of the telescope to f :2.7.

The field of view is ~ 7 arc min in diameter and a 5-cm diameter filter was used (this filter was centred at 6,567 Å with a full width at half maximum FWHM of 10 Å). The centre of the first selected field is at $\alpha = 1$ h 50 min and $\delta = -74^\circ 17'$ and falls on chart 58 V of the Hodge and Wright⁵ photographic atlas of the SMC, approximately between reference points WG 5 and L114. The scanning Perot-Fabry interferometer used interference order 798 (at H α), giving 8.2 Å, or 376 km s⁻¹ for the free spectral range. The observed field has been scanned with 21 steps (each step, or channel, corresponding to a given spacing of the plates of the interferometer) ensuring complete coverage of the free spectral range. Figure 1 shows the real-time display of this observation on a TV monitor. Four channels are selected

(number 1, 6, 11 and 16, clockwise from the upper left corner) and displayed on a 256 times 256 pixel screen while the full data (photon addresses in a 512 times 512 pixel grid for each of the 21 successive channels) are recorded on a magnetic tape. Each channel was exposed for 20 s in each scanning cycle of 21×20 s = 420 s. Then the cycle was repeated 25 times. Thus the total exposure time was 500 s for each of the 21 channels, with the seeing conditions fairly well averaged for each channel over the total exposure by the scanning mode used. The analysis of the data shows that the brighter interference rings on Fig. 1 are due to geo-coronal H α emission (the innermost one on channel 1 of Fig. 1) and OH night-sky line at 6569 Å (the precise wavelength⁶ of which is $6,568.72 \pm 0.02$ Å). The faintest ring (the one closest to the centre in channel 11 of Fig. 1) is due to faint H α emission by the object observed in the ultraviolet by the VWFC on board Spacelab 1. The possibility that it is produced by any night-sky emission line has been ruled out because it was not detected on the second field which was observed during the same night ($\alpha = 2$ h 10 min, $\delta = -74^\circ 34'$) despite an exposure time three longer (but only unscanned this time). The H α emission line profile that has been detected is broader and fainter than those of the night-sky emissions. The width of the line is as much as 60 km s⁻¹ compared with ~ 20 km s⁻¹ for the geo-coronal H α and for OH (the resolution imposed by the Finesse of the Perot-Fabry interferometer). The detected emission is fairly uniform over all the observed field and we may estimate its intensity by comparison with the geo-coronal H α emission line. Comparing interference rings of same diameter for the two lines, we calculated the ratio of the number of counts and applied a small correction ($\sim 15\%$) to take into account the fact that each line is transmitted through the interference filter with a slightly different transmission factor, because of its narrow bandpass. As a result we found an average intensity ratio H α (object)/geo-coronal H α = 0.4 ± 0.1 . Similarly, we found OH(6,569 Å)/geo-coronal H α = 0.8 ± 0.1 . The problem with the intensity of the night-sky lines is that they are highly variable, depending on parameters such as the geographical location, date, the time of night, zenith angle, the phase of the solar cycle and so on. Noting the values given by Chamberlain⁷, (5-20 Rayleighs for the geo-coronal H α emission at the zenith) and if we consider 10 Rayleighs as a typical value, we conclude that the detected object had an intrinsic emission around 4 Rayleighs. Converting into more usual units this gives an intrinsic monochromatic brightness around 10^{-6} erg cm⁻² s⁻¹, sr⁻¹ or 10^{-9} J m⁻² s⁻¹ sr⁻¹. This result is in very good agreement with the Johnson *et al.*² observation of the SMC-LMC bridge; they found a maximum H α brightness around $2-5 \times 10^{-9}$ J m⁻¹ s⁻¹ sr⁻¹ in the bridge between the two clouds.

However, the most interesting result is the radial velocity measured from the Doppler shift of the H α emission of this object. Comparing the diameters of the observed interference rings with calibration rings produced by a H α lamp we first

Fig. 1 Real-time display of four scanning channels on a TV monitor (geometric distortions making the rings non circular are due to the monitor itself). Each channel has been exposed 500 s. 21 channels insured a complete coverage of the field (clockwise from upper left are channel 1, 6, 11 and 16). The brighter interference rings are due to geo-coronal H α emission (the innermost one on channel 1) and OH 6,569 Å night-sky line (the second one from centre on channel 1). The H α emission of the SMC-LMC bridge appears as a faint ring surrounding to two bright rings of airglow on channel 1. Then one can see the diameter of the interference rings increasing while the interferometer is scanning from channel 1 to 21. The H α emission of the bridge appears as a large faint ring together with a small central spot on channel 6, a small central ring on channel 11, and the second ring on channel 16 (where OH is the central ring). The bright circular edge on each image is due to the less selective transmission on the edge of the interference filter.



checked the wavelengths of the geo-coronal H α emission and the OH night-sky line. The wavelength accuracy found for both lines is better than 0.05 Å (2 km s⁻¹ in radial velocity). The precision, indicated by the dispersion of the measured points, is about the same. The diameters of the H α interference rings from the object indicate an heliocentric radial velocity of 160 km s⁻¹ ± 5 km s⁻¹ all over the observed field (note that the rough value given by us in Courtès *et al.*³ was underestimated). Our final result is in very good agreement with the average H I velocities found by McGee and Newton⁸ and by Mathewson (personal communication)⁹ in the same area. Furthermore, they generally found several components (3–4) with velocities ranging from 140 to 200 km s⁻¹ and halfwidths around 20 km s⁻¹, which is consistent with the broadening observed for the H α emission. In some cases, our profile also appears to contain multiple velocity components but the signal-to-noise ratio remains too low to allow individual ones to be distinguished with certainty for their separations are near our spectral resolution.

It is clear, from the Doppler shift, that we are observing H α emission associated with the H I bridge between the SMC and LMC and that the large cloud of hotstars observed in ultraviolet light by the VWFC on board Spacelab 1 ionizes this bridge.

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The Sun's motion perpendicular to the galactic plane

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The period and amplitude of the Sun's motion perpendicular to the galactic plane are important parameters in some explanations of the terrestrial mass extinctions and cratering records^{1–5}. Here we have calculated the range of periods and vertical excursions that are consistent with the distributions of tracer stars in the Galaxy and have also evaluated the probable phase jitter in the solar period. We find acceptable half-periods for the vertical oscillation that range from 26 to 37 Myr (including the range of periods that have been inferred from the terrestrial records on mass extinctions and on cratering), maximum heights above the plane from 49 to 93 pc, and an average phase jitter per half-period of the order of 6–9%. The largest uncertainty in all these calculations is caused by the unknown distribution of the unseen mass that must be postulated to explain the distribution of observed stars^{6–7}. For all the models we consider, the most recent passage of the Sun through the galactic plane occurred in the past 3 Myr provided only that the present position of the Sun is between 0 and 20 pc above the plane. We extend the argument of Thaddeus and Chanan⁸ to show that the apparent periodicity in the mass extinction and cratering records cannot be caused by a population of objects (observed or unobserved) that contributes a major fraction of the total mass density at the solar vicinity.

We have calculated the time, t , that it takes the Sun to reach a height, z , above the galactic plane from the formula:

$$t = \int_0^z \frac{dx}{2(E - \varphi(x))^{1/2}} \quad (1)$$

where E is the total energy per unit mass of the Sun's vertical

motion and ϕ is the potential energy per unit mass. The major uncertainty in evaluating equation (1) arises from the distribution of the unseen matter that is inferred to exist at the solar vicinity^{6,7}. We have bracketed the allowed solar motion by using the potential ϕ calculated for two extreme Galaxy models, labelled here as the ISM and the all-disk models. In the ISM model, the unseen material is squashed towards the galactic plane to the maximum extent allowed by the analysis of the distribution of F dwarfs and of K giants; the postulated distribution is similar to that observed for ordinary interstellar material (hence the name). In the all-disk model, the unseen material has the largest exponential scale height (0.7 kpc) above the plane that is consistent with the observed rotation velocity of the galaxy near the solar position. In the all disk model, all of the unobserved material near the Sun is by construction in a flattened disk; there is no appreciable amount of unobserved material in a spherical halo. We also consider a more conventional model, the proportional model, in which the distribution of unseen material with a given velocity dispersion is proportional to the distribution of observed material, with the same velocity dispersion, the constant of proportionality being independent of velocity dispersion. The proportional model has approximately equal amounts of unobserved and observed material. The particular parameters we use for these models are given in Table 5 of ref. 7, rows 1 (proportional model), 10 (ISM model), and 12 (all disk model). For all three models, the potential was derived by solving self-consistently the Poisson and the Vlasov equations with realistic models for the Galaxy (typically 15 observed components).

Table 1 shows our results for the calculated half-periods and maximum heights above the plane of the solar vertical motion. For each of the models, we also list the time since the Sun last passed through the galactic plane. The characteristics of the calculated solar motion depend on the vertical velocity, v_{present} , and height above the plane, z_{present} . We give results for the range of values that are consistent with the available observational results⁹⁻¹¹, that is $v_{\text{present}} = 6-8 \text{ km s}^{-1}$ and $z_{\text{present}} = 0-30 \text{ pc}$. The most likely values are considered to be 7 km s^{-1} and 4 pc , respectively, although some determinations^{12,13} suggest that the Sun is 20-30 pc above the plane. The Sun's position with respect to the galactic plane could be determined more accurately using modern surveys of H I and CO.

The half-periods we calculate are: $31.5 \pm 1 \text{ Myr}$ (proportional

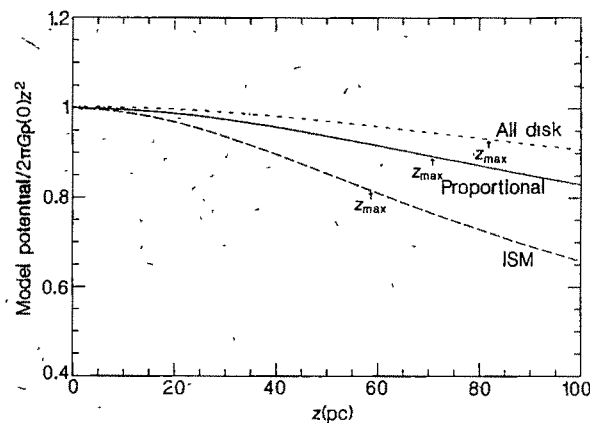


Fig. 1 The potential divided by $2\pi G\rho(0)z^2$ shown for the three numerical models.

model), $26.5 \pm 1 \text{ Myr}$ (ISM model), and $36 \pm 1 \text{ Myr}$ (all-disk model). The uncertainty due to the assumed current velocity and height above the plane is small compared with the difference between the values calculated with different distributions of the unseen material. For the most likely current parameters of the solar motion, the maximum height above the plane reached by the Sun, z_{max} , is: 70 pc (proportional model), 58 pc (ISM model), and 81 pc (all-disk model). The time since the last passage through the galactic plane is $< 3 \text{ Myr}$ in all the cases considered, provided that the Sun's current position is between 0 and 20 pc above the plane. The potential energy is currently much less than the kinetic energy for the solar vertical motion, so that the time since last passage is approximately the present height above the plane divided by the current velocity.

The problem of the solar vertical motion has usually been treated analytically by assuming^{8,14} that the potential for the vertical motion is quadratic in the height above the plane. In this approximation, the vertical motion is described by the equation for a simple harmonic oscillator. We show in Fig. 1 the potential, divided by $2\pi G\rho(0)z^2$, that was computed numerically for each of the galactic models we have discussed. Significant departures from the harmonic approximation are present beyond about 40 pc. Innanen *et al.*¹⁵ have evaluated numerically the solar motion in a simple galaxy model that includes departures from harmonicity.

How much of an error does the conventional assumption of a harmonic oscillator potential cause? The harmonic oscillator period differs from the exact half-period by 15% (4 Myr) for the ISM model, by 9% (3 Myr) for the proportional model, and by 6% (2 Myr) for the all-disk model. The error in the maximum height above the plane implied by the harmonic oscillator potential is, for the same three cases, 11, 6 and 4%.

The harmonic oscillator approximation can be used with relatively small errors in the computation of the fractional amount of time the Sun spends in any specified height range, once the period and maximum height above the plane have been calculated from the exact potential. We have evaluated numerically the fraction of time that the Sun spends in various height intervals using the potentials shown in Fig. 1 for the three illustrative galaxy models and have compared the results with the analytical formula¹⁴ that applies in the case of a harmonic oscillator. Adopting the exact values of the half-period, $P_{1/2}$, and of z_{max} , we find that the curve of elapsed times is described to an accuracy of 3% by the harmonic oscillator relation: $t(z)/P_{1/2} = \frac{1}{\pi} \arcsin(z/z_{\text{max}})$.

Some explanations⁵ attribute the apparent periodicities in the extinction data to phenomena occurring at the apex of the solar vertical motion. These explanations are unlikely to be correct because they would require an extinction event that lasts a time ΔT to occur all within $7 \text{ pc} \times (\Delta T/\text{Myr})$ of z_{max} .

How much phase jitter would one expect in the times at which the Sun crosses the galactic plane? We model the (unspecified) process that causes stars to have velocity dispersions that

Table 1 The Sun's vertical oscillation

Model	$\rho_{\text{total}}(z=0)$ ($M_{\odot} \text{pc}^{-3}$)	v_{present} (km s^{-1})	z_{present} (pc)	Half-period (Myr)	Last passage (Myr)	z_{max} (pc)
Proportional	0.21	7	0	31.4	0.0	70.0
		7	4	31.4	-0.6	70.1
		7	10	31.4	1.4	70.9
		7	20	31.6	2.7	73.4
		7	30	31.8	3.9	77.4
		7	40	32.1	5.0	82.6
		8	4	32.0	0.5	81.2
		6	4	30.8	0.6	59.3
ISM	0.33	7	0	26.5	0.0	57.8
		7	4	26.5	0.6	58.0
		7	10	26.6	1.4	59.0
		7	20	26.9	2.7	62.5
		7	30	27.5	3.8	67.9
		7	40	28.1	4.8	74.6
		8	4	27.5	0.5	68.0
		6	4	25.6	0.6	48.6
All disk	0.15	7	0	36.1	0.0	81.1
		7	10	36.1	1.4	81.8
		7	20	36.2	2.7	83.9
		7	30	36.3	4.0	87.2
		7	40	36.4	5.2	91.6
		8	4	36.5	0.5	93.6
		6	4	35.7	0.6	69.0

increase with age by calculating simulated orbits in which a model star receives a vertical kick in velocity, δv , of random sign every half period. Solar type stars have¹⁶ a z -velocity dispersion of the order of 20 km s^{-1} after 5,000 Myr (the solar age), indicating that the Sun currently has an unusually small velocity perpendicular to the plane for a star of its age. To model what happens to typical solar-type stars, we adopt $\delta v \approx 20 \text{ km s}^{-1}/(\text{solar age}/P_{1/2})^{1/2} \approx 1.6 \text{ km s}^{-1}$. For anharmonic potentials like those shown in Fig. 1, the phase jitter per half-period, $\Delta\phi$, can be estimated analytically to be of the order of

$$\Delta\phi \approx \frac{0.2\delta v P_{1/2} n^{1/2}}{z_{\text{anh}}} \quad (2)$$

where z_{anh} represents approximately the anharmonicity of the potential in the relation $\phi(z) \propto z^2/[1+(z/z_{\text{anh}})]$ and n is the number of elapsed half-periods. In deriving equation (3), we have assumed that $z_{\text{max}} \ll z_{\text{anh}}$ and $\delta v \ll v_{\text{present}}$. For an elapsed time of 250 Myr, $\Delta\phi \approx 0.8 \text{ kms}^{-1} P_{1/2}/z_{\text{anh}}$. Values for z_{anh} can be estimated from Fig. 1; the half-periods, $P_{1/2}$, are listed in Table 1. For a harmonic potential, the phase jitter per half-period is zero if the velocity kick always occurs when the Sun crosses the galactic plane; the jitter decreases like $1/n^{1/2}$ if the kicks occur with equal probability between 0 and 40 pc.

We have carried out a series of Monte-Carlo simulations of the solar vertical motion including random kicks that are given with either a uniform probability between 0 and 40 pc or always in the plane. The results are shown in Table 2. The Monte-Carlo

Table 2 Expected phase jitter for solar period from vertical kicks in velocity

Model	Location of kick (pc)	$\Delta\phi$ (median)	Quartiles
Proportional	0.0	0.035	± 0.02
	0-40	0.045	± 0.03
ISM	0.0	0.07	± 0.035
	0-40	0.07	± 0.04
All disk	0.0	0.02	± 0.01
	0-40	0.03	± 0.02

simulations are in good agreement with the analytical estimate given in equation (3). The typical phase jitter after 250 Myr is in the range 0.03–0.07 per half-period, depending on the Galaxy potential that is adopted.

The vertical oscillation period is also changed by the epicyclic motion of the Sun in the plane of the galaxy. Let the half-amplitude of the epicyclic motion be X_{max} and let h be the exponential scale length of the disk density in the plane. The average period is only slightly affected by the epicyclic motion,

$$\text{Period}(X_{\text{max}}) \approx \text{Period}(X_{\text{max}}=0) \times \left[1 + \frac{X_{\text{max}}^2}{12 h^2} \right] \quad (3)$$

The average correction due to the epicyclic motion is $<1\%$ for typical values^{11,17} of $h(3.5 \text{ kpc})$ and $X_{\text{max}}(0.5 \text{ kpc})$. However, for times short compared with the period ($\approx 160 \text{ Myr}$) of the solar epicyclic motion in the plane of the Galaxy, there is also a sinusoidal correction to the vertical oscillation period of amplitude $X_{\text{max}}/2h$. This correction could, in principle, be taken into account in making comparisons with observational data provided that the phase of the Sun in its epicyclic motion is not changed drastically during the time under consideration. On the other hand, the simplest (and most conservative) procedure is to ignore, when comparing with observations, the dependence of the period on the solar position in the galactic plane and to compute the total expected phase jitter by combining quadratically the previously computed jitter in the vertical motion (see Table 2) with an apparent phase jitter from the epicyclic motion in the plane. In this case, the expected r.m.s. phase jitter arising from the motion in the plane is $X_{\text{max}}/2^{3/2}h \approx 5\%$, comparable with the phase jitter caused by vertical kicks in velocity

The combined calculated phase jitter per vertical half-period is in the range 6–9% for all the models we have considered. This calculated phase jitter is smaller than the observed jitter in the biological and cratering data.

Finally, we note that interactions with the unseen material cannot have induced the approximately periodic features that are inferred^{1–4} from the extinction and cratering data. This conclusion follows from the work of Thaddeus and Chanan⁸, who showed that molecular clouds are not sufficiently concentrated towards the plane of the Galaxy to cause a strong preference for interactions at small heights. The unseen material cannot be more concentrated towards the plane than are molecular clouds. The maximum concentration of the unseen material towards the galactic plane that is consistent with the observations of F dwarfs and K giants is represented by the ISM model^{6–7}, in which the unseen material has an exponential scale height which is like that of the molecular clouds (corresponding to a velocity dispersion of 4 km s^{-1}). Even in this extreme model, the maximum height above the plane reached by the Sun (49 pc) is about equal to the height (45 pc) at which the density of unseen material drops to e^{-1} of its value in the plane. The Sun encounters unseen material everywhere in its orbit, with only a moderate preference for interactions in the galactic plane.

All the observed components of the Galaxy that constitute major fractions of the mass density in the solar vicinity have scale heights that are comparable with or larger than the maximum heights, z_{max} , for the Sun listed in Table 1. If something associated with passages through the galactic plane is causing periodic mass extinctions and cratering, then whatever it is cannot constitute a large fraction of the mass density, either observed or unobserved.

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Increased concentration and vertical distribution of carbon dioxide in the stratosphere

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Although many measurements of the abundance of CO_2 in the troposphere have been made, knowledge of its stratospheric abundances and variability is sparse. Here we report mid-latitude vertical profiles of CO_2 , up to 35 km, measured in 1979, 1982 and 1984 by analysing cryogenically collected balloon samples supplemented by air samples taken aboard aircraft. CO_2 mixing ratios are not constant with altitude but rather decrease by $\sim 7 \text{ p.p.m.v.}$ (parts per 10^6 by volume) from the tropopause to the mid-stratosphere.

The growth rate of the atmospheric CO_2 abundance caused by anthropogenic emission, which varies between 1.0 and 1.5 p.p.m.v. yr^{-1} at ground level¹, is also observed at all stratospheric heights up to 35 km. The shape of the profiles suggests that excess CO_2 above 20 km enters the stratosphere through tropical upwelling rather than mid-latitude diffusion. The time lag of this height region with respect to the tropospheric CO_2 level is ~ 5 yr.

Cryogenic sampling provides a reliable technique for collecting large stratospheric whole air samples. With the Max-Planck Institut für Aeronomie (MPAE) balloon-borne cryogenic samplers using liquefied neon as a refrigerant, samples of 30 l STP can be collected in 500 cm^3 tubes, at 8 and 15 different heights, respectively. Subsequent gas-chromatography analyses allow for simultaneously determining the concentration of 23 stable source gases, such as H_2 , CH_4 , N_2O , CO and various halocarbons. Vertical profiles of these trace constituents, up to 35 km in altitude, have already been determined in this way²⁻⁴.

For CO_2 , the infrared absorption gas analysis provides a more precise analytical technique than gas chromatography; flask samples can be analysed with a total error of $\sim \pm 0.2$ p.p.m.v. (ref. 1). This method was applied to samples collected during 1977-79 balloon flights. The profiles obtained⁵ show CO_2 volume mixing ratios decreasing from a tropopause level to a mid-stratospheric level above 20 km, by ~ 7 p.p.m.v. this decrease taking place almost entirely within the 15-20 km altitude range.

It was found, however, that these first measurements, in particular those related to 1977 and 1978 balloon samples, had suffered from contamination problems because the sample aliquots had been stored for >1 yr. Moreover, the pressure of most samples was <5 bar; for such low pressures, repetitive analyses had shown that CO_2 concentrations can drift considerably. Thus, CO_2 data analysed from most 1977/78 samples had to be discarded. Samples from two flights in June and November 1979 were analysed with a delay of four months, but still several sample aliquots had a pressure <5 bar resulting in some scatter of the data. Figure 1 shows those of the 1979 CO_2 data analysed from stratospheric samples of >5 bar. Supplementary tropospheric CO_2 data analysed from samples collected aboard commercial airliners during June and November 1979 at mid-latitudes are also shown.

On 20 September 1982, 14 stratospheric samples were recovered from a balloon flight of the MPAE 15-tube sampler, at Aire-sur-l'Adour, France (44°N). Supplementary samples were collected on 23 September aboard Concorde between Paris and New York and aboard a Boeing 747 during the return flight on 25 September. Two years later, on 1 October 1984, a tandem flight of the 8-tube and the 15-tube cryogenic samplers, at Aire-sur-l'Adour, yielded 22 samples almost equally spaced between 10 and 35 km in altitude. Supplementary aircraft samples were collected on 20 September between Stockholm and Kiruna and on 4 October between Stockholm and Frankfurt.

All analyses of the 1982 and 1984 balloon samples were made without aliquoting, that is, the original sample air was transferred directly to the gas analyser. The absolute calibration was achieved with the aid of standard gases belonging to the Stockholm calibration gas system. These gases, consisting of mixtures of CO_2 in air and frequently being compared with the calibration system at Scripps Institution of Oceanography (SIO), are related to the '1974 adjusted index' calibration scale⁶. Corrections are needed for the carrier gas effect (SIO used CO_2 in N_2 mixtures) as well as for non-linearity and drift in the SIO calibration system. Because of uncertainties that still exist in the interpretation of calibration results we present our data in the 1974-adjusted index scale. We realize that true concentrations, within the range of CO_2 values reported here, are higher by $\sim 4.0 \pm 0.3$ p.p.m.v. With sample sizes ranging between 20 and 30 l STP, the CO_2 analyses were made within two months after the flights, when sample pressures ranged between 40 and 60 bar. Repetitive analyses made two months later showed no changes of the concentrations that exceeded the error limits of ± 0.2 p.p.m.v. The 1982 and 1984 stratospheric profiles of CO_2 displayed in

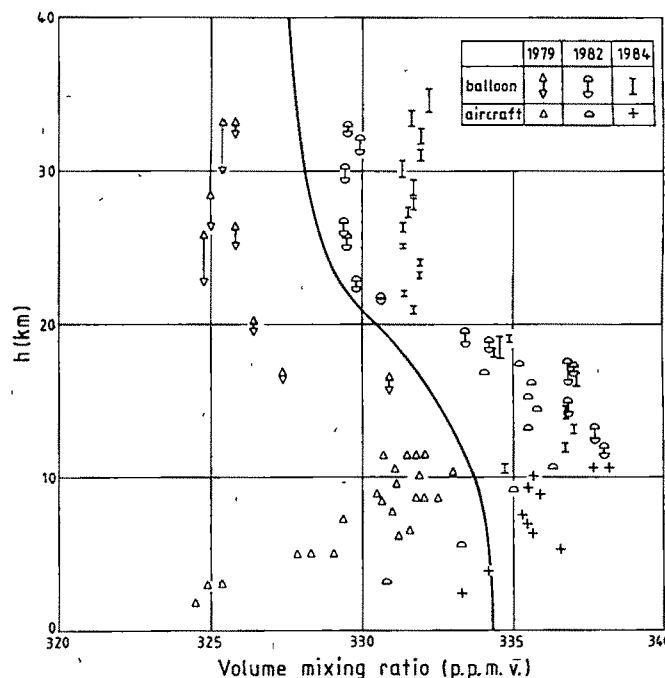


Fig. 1 Vertical distribution of CO_2 between the ground- and 35-km altitude as analysed by infrared absorption of whole air samples collected aboard balloon and aircraft platforms, for 1979, 1982 and 1984. The height range of the balloon samples is shown by the symbols. The modelled profile (solid line) computed by means of a one-dimensional time-dependent model corresponds to 1980 conditions.

holds for the aircraft samples analysed immediately after the flights.

The 1982 and 1984 CO_2 profiles confirm, in general, the existence of a decreasing CO_2 content with elevation. Even more important, they confirm that this decrease is confined to the height region between 15 and 20-22 km. The stratospheric CO_2 decrease begins ~ 5 km above the tropopause which, according to aerological soundings at nearby Bordeaux meteorological station, was located at 10, 11 and 12 km during the 1979, 1982 and 1984 balloon flights, respectively. Above 20-22 km, the CO_2 mixing ratio is almost constant with altitude; the 1984 profile even shows an indication of a slight increase above 30 km.

A striking feature of the CO_2 profiles presented here is the overall similarity of the stratospheric portions above 20 km. Obviously, the general increase of the tropospheric abundance of CO_2 resulting from the burning of fossil fuel is reflected by a stratospheric increase at a corresponding rate. Mid-stratospheric mixing ratios, as averaged over the height range above 22 km, are 325.4 ± 0.5 , 329.6 ± 0.2 , and 331.6 ± 0.3 p.p.m.v., for 1979, 1982 and 1984, respectively. Annual increase rates thus amount to 1.4 p.p.m.v. yr^{-1} between 1979 and 1982 and 1.0 p.p.m.v. yr^{-1} between 1982 and 1984 or an average of 1.2 p.p.m.v. yr^{-1} over the whole time range discussed here. These rates are comparable with those observed at tropospheric levels^{1,7}.

Annual means of tropospheric CO_2 as obtained from the Stockholm project, for the years discussed here, were found to be in excess of 6.8 ± 0.9 p.p.m.v. over the stratospheric mixing ratios reported here corresponding to a time lag of 5.2 ± 0.8 yrs. The tropospheric CO_2 profiles shown in Fig. 1 are representative of late summer/autumn conditions, when the cumulative uptake of CO_2 by the vegetation reaches its maximum, thus in the annual cycle a minimum is obtained in August/September. In late winter/spring, when CO_2 is returned to the atmosphere, a maximum occurs in April/May. This seasonal variation, having a total amplitude of ~ 7 p.p.m. in the northern upper troposphere, is almost undetectable within the lower stratosphere¹.

the annual mean occurs in September. Thus, the results obtained from the lower stratosphere during autumn as shown in Fig. 1 may represent maximum rather than average conditions.

Compared with the fairly regular behaviour of mid-stratospheric profiles, the transition region below, between ~22 km and the tropopause, seems to reflect more complex features. The 1982 balloon profile, for instance, reveals exceptionally high CO₂ mixing ratios between 12 and 17 km that are comparable with or even higher than the corresponding 1984 data. As shown by the laminar structure of observed ozone profiles, transport in the lower stratosphere is largely achieved by large-scale quasi-horizontal advection. Near the tropopause, direct tropospheric/stratospheric mixing, often in connection with tropopause folding, may also be important. Either of these processes may be responsible for the high CO₂ values observed in 1982. Although some deviations between balloon and aircraft sample results exist, which are likely to result from different sampling locations, the CO₂ content below 16 km, in each of the 1982 and 1984 vertical distributions is nearly unchanged within this lower part of the stratosphere. There is a distinct change of the CO₂ mixing ratio from this layer towards the upper troposphere. This shift, as well as the rather uniform vertical distribution of CO₂ within the lower stratosphere, agrees with previous results of the Stockholm project⁸.

Our results show that stratospheric CO₂ concentrations increase with time at about the same rate as tropospheric abundances. The shape of the profiles, however, suggests that excess CO₂ from anthropogenic sources which are concentrated at northern mid-latitudes, does not simply propagate upwards there. A theoretical CO₂ profile computed for 1980 conditions by means of a time-dependent one-dimensional model, simulating vertical diffusion and following the observed growth of CO₂ at ground level³, clearly demonstrates that the observed profiles

cannot be reproduced by vertical diffusion alone. The fact that the mixing ratio gradient is confined to the height region between ~15 and 20 km and that CO₂ above that height is rather constant with altitude indicates that excess CO₂ must have entered the mid-stratosphere in a different way. We suggest that tropical upwelling is the mechanism by which the required vertical transport is probably achieved. It may carry CO₂ from the tropical troposphere to the middle and high tropical stratosphere, from where it spreads polewards. At all levels between 22 and 35 km, excess CO₂ obviously arrives with the same delay of ~5 yr with respect to the troposphere. The slight CO₂ increase above 30 km indicated by the 1984 profile suggests that the delay time for this upper region may even be shorter.

In this respect, measurements of stratospheric CO₂ profiles at tropical latitudes would be of great importance. Note that the existence of a shaped CO₂ profile as shown by our data may be relevant for satellite sounders that use the assumption of well-mixed CO₂ in the stratosphere to retrieve temperatures from infrared spectral features.

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Rate of sulphur dioxide emission from Erebus volcano, Antarctica, December 1983

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Mount Erebus is the principal source of volcanic emanations to the atmosphere in Antarctica. The rate of emission of SO₂ from Mt Erebus was determined to be 230 ± 90 tonnes day⁻¹ during an 11-day period in December 1983. This is a more accurate and much higher estimate than previous ones. Because of the very constant character of the volcano's activity from the earliest observations until September 1984, the emission rate is possibly representative of the past 20 yr. The new data are important for accurate assessment of volcanic input to the clean Antarctic atmosphere, and are a useful baseline for the changes in emission rates which will be measured now, after Erebus has begun a new energetic eruptive period.

Mt Erebus is the only active and the largest of three volcanoes which form the bulk of Ross Island, in the Ross Sea (77.55° S, 167.17° E). It is made up chiefly of anorthoclase phonolite lavas and has sustained an active lava lake, at least since 1972, and possibly for more than a century. Most of the observations of Erebus cite a persistent volcanic plume which emanates from its summit crater¹. Beginning in September 1984, Erebus has begun to exhibit much more explosive and prolific activity. The December 1983 period represents the low-level activity which

prevailed before 1984, and, therefore, represents a kind of baseline against which later observations, made during the more prolific activity, can be compared. Previously published estimates of the rate of emission of SO₂ by the Erebus plume have been 3 tonnes day⁻¹ in 1978 (ref. 2), and 35 tonnes day⁻¹ in 1980 (ref. 3). Interest in measurements of SO₂ and particle fluxes comes not only from volcanologists wishing to use such measurements to interpret the behaviour of the volcano, but also, particularly in the case of Erebus, from those trying to establish the sources of sulphate aerosol accumulations in the remote Antarctic environment⁴. Our objective was to make simultaneous and accurate airborne measurements of the gas and particle fluxes in the Mt Erebus plume. This is a report on the SO₂ measurements; we will discuss the particle data elsewhere.

To make measurements of the SO₂ fluxes, a correlation spectrometer (Barringer COSPEC-V) and an aircraft (C-130) survey method⁵ were used. The instrument is directed upwards and measures absorption of ultraviolet (290-300 nm) skylight, as the plane traverses under the plume, perpendicular to its dispersal axis. Such measurements give excellent estimates of the cross-section of the SO₂ burden, which must be multiplied by the wind speed to give the emission rate. On board the C-130 we were aided by continuous inertial navigation system readings of wind speeds (usually ~50 readings per traverse). Thus the largest source of error in such measurements was controlled very well compared with similar studies. Because of this, we believe our SO₂ flux estimates are of the highest quality. The previous estimates^{2,3} used much less direct methods and are more error-prone. Because the COSPEC measures only SO₂, the estimates obtained by this method are less than the total S flux of the volcano, because S is also produced as H₂S and as liquid and solid aerosol particles in volcanic plumes.

The general atmospheric conditions for sampling and measuring the plume on all four dates (9, 14, 17 and 19 December) were good, with clear visibility and light to moderate winds. Results of the individual SO₂ flux measurements and the measurement conditions of each of the traverses we made are

Table 1 SO₂ flux determinations, Erebus volcano, December 1983

Traverse	Distance downwind (km)	Plume width (km)	Mean SO ₂ concentration (p.p.m.v.)	SO ₂ (tonnes per day)
a, 9 December 1983. Plume thickness, 300 m; winds SE at 5–7 knots				
1	3	6.6	0.30	160
2	6	13.5	0.26	360
3	6	14.4	0.26	440
4	6	14.2	0.26	440
5	3	6.0	0.26	170
6	3	9.3	0.18	150
1–6		$\mu \pm \sigma$		290 ± 140
b, 14 December 1983. Plume thickness, 100–200 m; winds W at 25–28 knots				
1	2	2.4	0.13	135
3	2	3.9	0.11	169
4	2	3.3	0.12	177
5	2	4.2	0.11	203
6	2	2.7	0.13	164
7	2	4.2	0.07	120
8	2	3.3	0.08	115
1, 3–8		$\mu \pm \sigma$		154 ± 32
c, 17 December 1983. Plume thickness, 300 m?; winds W at 8–10 knots				
1	3	8.7	0.10	242
2	3	8.1	0.13	295
3	3	9.0	0.10	306
4	3	8.7	0.08	221
5	3	10.2	0.07	240
6	3	10.5	0.11	414
7	3	10.2	0.10	302
1–7		$\mu \pm \sigma$		289 ± 65
d, 19 December 1983. Plume thickness, 150–200 m; winds WNW at 8–12 knots				
1	3	5.1	0.20	211
2	3	4.8	0.11	111
3	3	4.8	0.19	191
4	3	4.5	0.18	163
5	4	7.5	0.17	205
6	3	3.9	0.27	219
7	3	4.2	0.24	206
1–7		$\mu \pm \sigma$		195 ± 48

listed in Table 1. The wind conditions were variable and the geometry of the plume varied considerably throughout the period 9–19 December. Concentrations of SO₂ within the plume, based on the burden measurements and width and thickness data for the plumes, were in the range 0.07–0.30 p.p.m.v. (parts per 10⁶ by volume).

On the 9 December survey a fortunate event occurred. The plume, which from a distance had appeared white and billowy was momentarily darker in colour as we approached the volcano, 20 min before the survey. This darkening of the plume was probably due to a brief (1–2 min) strombolian explosion within the crater. Similar events have been described by Kyle *et al.*¹. Considering the timing of this brief explosion, the wind speed and the times of our COSPEC traverses, it is likely that the higher fluxes of traverses 2–4 (Table 1a) are due to this

explosion. It is also probable that this event affected our particle-size distributions, which were determined at a greater distance from the volcano after the COSPEC survey. The frequency of small explosions can be estimated from seismic disturbances which are correlated with them (J. Kienle, personal communication; based on reading the seismic records of the Erebus network at Scott Base) and seems to be about 1–3 per day. Because such explosions are infrequent, and probably last only a few minutes, we suggest that they do not increase the daily flux of SO₂ by more than a few per cent, even though the rate is several times higher while the explosions occur.

Table 2 summarizes the 4 days of SO₂ flux determinations at Erebus and demonstrates the overall average of 230 ± 90 tonnes day⁻¹. Considering our knowledge of the typical behaviour of Erebus, we believe this is a good estimate of the steady-state SO₂ emission of the volcano. Because we were fortunate to make measurements during one strombolian eruption, we can show that the occasional occurrence of such events does not affect the average flux significantly. The 1983 rate of emission for Erebus is lower than most volcanoes in an active eruptive state, which frequently have been estimated to emit >1,000 tonnes day⁻¹ (refs 6, 7).

Using the SO₂ emission rate it is possible to calculate the volume of the degassing magma chamber, by the approach of Rose *et al.*⁸. Electron microprobe analyses of glass inclusions in anorthoclase phenocrysts indicate a pre-eruptive sulphur content in the phonolitic magma of 440 p.p.m. (P.R.K., unpublished data). The sulphur content of whole rock samples ejected from the lava lake averages 260 p.p.m. (P.R.K., unpublished data). Assuming a magma density of 2.7 g cm⁻³ and 20 yr of degassing, a minimum magma volume of 1.7 km³ would account for the observed SO₂ emission rate. For 80 yr of degassing the volume of magma is 6.9 km³. Although Mt Erebus has been characterized by a large vapour plume throughout this century¹, the observations before the 1960s are particularly sparse and the calculated volume of 6.9 km³ appears to be unrealistically high. We prefer the 20-yr degassing volume of 1.7 km³. This is consistent with observations which indicate no significant change in the degassing and volcanic activity of Mt Erebus since the 1960s. Also, an aerial photograph taken in November 1963 indicates the existence of a small lava lake similar to that which existed from 1972 to 1982 (ref. 1).

The sulphur content of 440 p.p.m. in the Mt Erebus anorthoclase phonolite magma is low compared with reported concentrations of 800–2,800 p.p.m. S in basaltic and andesitic glass inclusions^{9–11}. The release of the sulphur from the magma, either in the form of H₂S and/or SO₂, will be a function of several variables, including oxygen fugacity¹². Estimates of sulphur and oxygen fugacity in the phonolite of log $F_{S_2} = -2.7$ and log $f_{O_2} = -12.2$ at 1,000 °C have been made of phonolite samples using the composition of pyrrhotite and other mineral phases¹³. In such conditions the fugacity of H₂S is likely to be high¹². One would therefore predict significant emission of H₂S in the Erebus plume. Analyses of gases within the phonolite glasses show a SO₂/H₂S ratio of ~2 (E. Bigelow and P.R.K., unpublished data). Very few accurate measurements of H₂S have been made in volcanic plumes⁷ and the rate of H₂S oxidation ultimately to sulphate particles is unknown for the Antarctic environment. However, H₂S must not be ignored when assessing the contribution of Mt Erebus to the atmospheric sulphur budget

Table 2 Summary of Table 1

Date (December)	Distance (km)	Width (km)	Concentration (p.p.m.v.)	Tonnes per day (means ± σ)	Winds (knots)	No. of traverses
9	3–6	6–14	0.18–0.30	290 ± 140	5–7, SE	6
14	2	2.4–3.9	0.07–0.13	150 ± 30	25–28, W	7
17	3	8–10.5	0.07–0.13	290 ± 65	8–10, W	7
19	3	3.9–7.5	0.11–0.27	200 ± 50	8–12, WNW	7
	Overall $\mu \pm \sigma$			230 ± 90		27

for Antarctica. We are unable to quantify this contribution at present.

Our 1983 estimate of SO_2 emission from Mt Erebus is substantially higher than previous measurements. It is probable that Mt Erebus contributes significant amounts of sulphur to the Antarctic atmospheric sulphur budget. Radke³ suggested that Erebus contributed 32% of the sulphate to the budget. Delmas⁴, however, suggested that the effect of Erebus was small. Our measured SO_2 emission, excluding the possible contribution of H_2S , represents ~10% of the total Antarctic sulphur budget as estimated by Delmas⁴. Erebus is unlikely to influence the atmosphere over all of Antarctica, but its local effect may be significant. Because of the prevailing winds much of the sulphur is probably removed as aerosol over the Ross Sea, to the north of Erebus. However, as Delmas⁴ pointed out, the sulphur may be transported over large distances. Ultimately, it could circulate over the West and East Antarctic ice sheets. In addition, Erebus may contribute to the Antarctic stratosphere, especially during the winter months when the tropopause is significantly lower. A careful examination of the Antarctic sulphur budget, bearing in mind the Erebus contribution, is needed.

Because the activity of Erebus has been remarkably invariable in the period of human observations¹, we expect that the SO_2 emission rate we measured may represent a modern pre-1984 average for that volcano, which may be applied to the 1972–83

period and possibly for longer. We hesitate to conclude that the lower estimates of SO_2 flux in 1978 (ref. 2) and 1980 (ref. 3) show that the SO_2 emissions were increasing in late 1983 as a precursor of the increased activity, because the earlier estimates were made by very different methods. But the possibility that this does represent a precursive increase in SO_2 is consistent with experience at other volcanoes^{5,6}. We expect to continue SO_2 emission measurements throughout the current renewed activity to record the pattern and quantity of SO_2 release there.

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Direct measurement of air and precipitation particle motion by very high frequency Doppler radar

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Microwave meteorological Doppler radars are the most efficient tool for understanding the dynamical properties of precipitating systems in the troposphere¹. However, these radars do not directly observe ambient air motion, but rather the velocity of precipitation particles within a particular region. We have recently completed very high frequency (VHF) Doppler radar at Shigaraki, Japan (34.85° N, 136.10° E) which has enabled the three-dimensional motion of both air and precipitation particles to be observed simultaneously. In particular, it enables vertical air motion as well as precipitation fall speed to be directly observed. Doppler broadening of the precipitation echo reveals features which, when above the melting layer, are characteristic of snowflakes and, when below this layer, are characteristic of raindrops. Sensitive VHF (and, most likely, ultrahigh frequency, UHF) Doppler radars equipped with fast-beam steerability open new possibilities for the investigation of the dynamical properties of precipitation particles in close combination with ambient air motion.

MST (mesosphere-stratosphere-troposphere) radars are sensitive, high-power VHF/UHF Doppler radars that can detect clear-air (refractive-index) fluctuations or turbulence in the middle atmosphere (altitude range of 10–100 km^{2,3}). Received echoes are Doppler shifted because of radial motion of the clear air turbulence (CAT). The mean Doppler shifts acquired in three different directions provide three-dimensional velocity of CAT. This velocity is shown to be equal to the ambient air motion by comparison with measurements obtained by conven-

tion^{2,4}. MST radars have rarely been used for the observation of tropospheric meteorological phenomena, with the exception of a few short-term studies of frontal zones⁵ and thunderstorms⁶.

Radar reflectivity of the inertia subrange turbulence in clear-air regions is dependent on the power law of $\lambda^{-1/3}$ (where λ is the radar wavelength). The reflectivity in the VHF band is observed to be within the range of 10^{-18} – 10^{-15} cm⁻¹ in the troposphere and lower stratosphere⁷. The power law yields a reflectivity of 5×10^{-18} – 5×10^{-15} cm⁻¹ in the microwave frequency band.

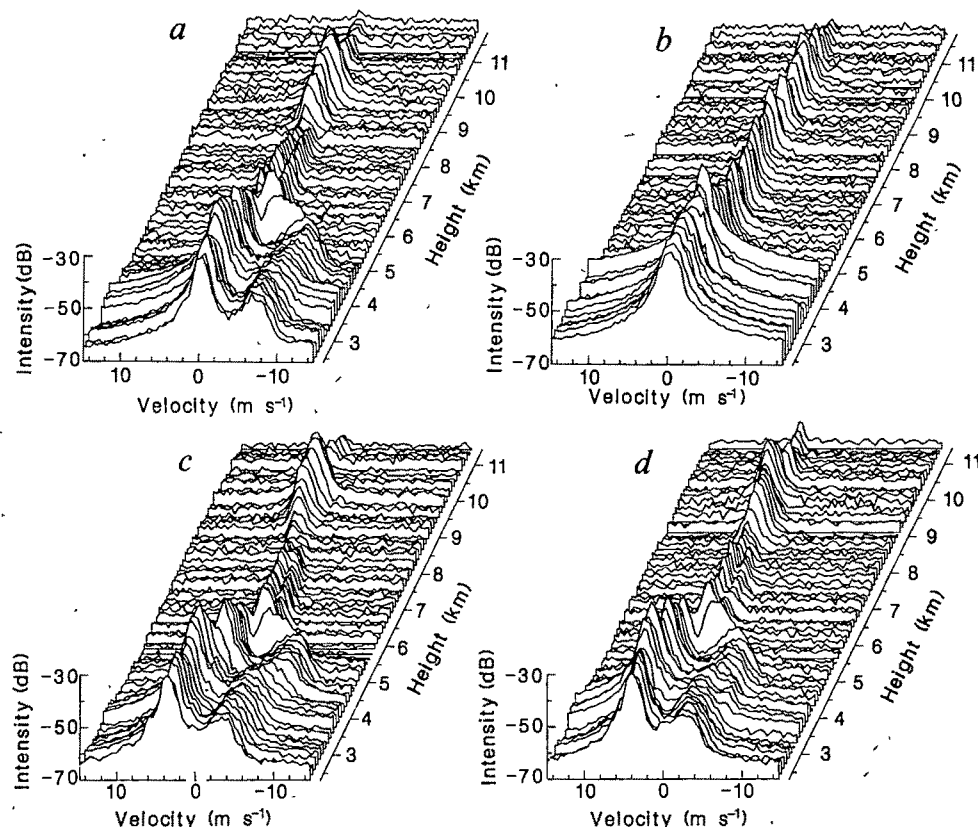
On the other hand, an estimate of the radar reflectivity for raindrops in the VHF band, for example, at 50 MHz, gives 10^{-19} – 10^{-16} cm⁻¹ for a precipitation rate of 3×10^{-1} – 3×10 mm h⁻¹, assuming the Marshall–Palmer drop-size distribution function (T. Yokoyama, personal communication). The reflectivity of the melting layer (or bright band) is ~50 times larger than that of raindrops. Thus, the reflectivity of precipitation particles at 50 MHz is within the range of 10^{-19} – 5×10^{-15} cm⁻¹, which is essentially the same range as that of CAT. This value is nine orders in magnitude smaller than that of microwave radars.

Because the reflectivities of both CAT and precipitation particles at 50 MHz are in the same range, sensitive 50-MHz MST radars are expected to detect simultaneously both types of echoes from precipitating air. This contrasts with the situation found in microwave radars, where precipitation echo is much more dominant than CAT echo. This is because the microwave reflectivity of precipitation particles is 4–12 order of magnitude larger than that of CAT.

The middle and upper atmosphere radar (MU radar) located at Shigaraki, Japan is a 46.5-MHz radar using an active-phased array system⁸. It is composed of 475 Yagi antennas and an equivalent number of solid-state power amplifiers (transmitter-receiver (TR) modules)⁹. Each Yagi antenna is driven by a TR-module with peak output power of 2.4 kW. The nominal peak and average radiation powers of the whole system are 1,000 and 50 kW, respectively. This system makes it possible to steer the antenna beam up to 30° from the zenith in each interpulse period.

Recently, we have observed precipitating air in the troposphere, using the fast beam steerability of the MU radar. We present the preliminary results of a short-term study which was successfully conducted on 22 August 1984. The full capabilities of the MU radar system were only partially employed. A nominal beam width of 4.0° and peak transmitted power of 760 kW were

Fig. 1 Doppler velocity spectra plotted against altitude obtained in the vertical direction by the MU radar, Shigaraki, Japan. The observational periods are: *a*, 0845–0851 JST (Japan Standard Time) and *b*, 0750–0756 JST on 22 August 1984. The ordinate is relative power in decibels with an arbitrary reference level. The abscissa is represented in terms of radial (vertical) Doppler velocity (Note that the sign is given in the reverse direction.) The positive and negative speeds correspond to departure from and approach to the MU radar, respectively. This usage is the opposite of the meteorological convention. *c*, *d*, Same as *a*, except *c* is northward and *d* is the eastward beam direction. The spectra are plotted against radial Doppler velocity.



resolution of 150 m. Echoes were sampled above 2.4 km from ground level (375 m above sea level) to obtain the delay required to protect the TR modules. The beam was steered during each interpulse period of 400 μ s sequentially to three different directions: zenith and 15° away from the zenith to the north and east. At an altitude of 5 km, the distance separating the off-vertical beams from the vertical beam was 1.3 km.

The bipolar video signal from each beam was sampled at 64 points spaced in the region from 2.4 to ~ 12 km aloft at 150-m intervals. 128 point complex fast Fourier transforms were calculated in real time to obtain Doppler velocity spectra for each 7.7-s period. The resulting power spectra were averaged for ~ 1 min before being written on magnetic tape.

Figure 1*a, b* shows typical altitude variations of the Doppler velocity spectra obtained in the vertical direction during periods (*a*) with and (*b*) without perceivable precipitation on the surface. Each spectrum is the average of a 6-min period. The surface rainfall rates during these periods were 1 and 0 mm h^{-1} , respectively, as observed by the Japan Meteorological Agency at their

facility in Kinose, 6.9 km north of the MU radar. The rainfall is classified as being of the weak stratiform type.

Of the spectral components illustrated in Fig. 1, the minor one with comparably large negative (downward) Doppler shift exists only when precipitation is observed (Fig. 1*a*), whereas the major one with near zero Doppler shift appears persistently irrespective of precipitation.

Figure 1*c, d* clearly illustrates the correspondence of these spectral components to those obtained in the northward and eastward beam directions. For the major spectral component, horizontal velocity is calculated by assuming the presence of a uniform velocity field within the region illuminated by the three beams (Fig. 2). The result is compared with the horizontal wind velocity measured by rawinsondes launched from Shionomisaki, ~ 150 km south of Shigaraki, and from Wajima, 250 km north. The radar-deduced horizontal velocity is consistent with the meteorological wind field, the difference between the three locations is in accord with that usually observed below 12 km (ref. 9). The vertical velocity shown in Fig. 2 is directly measured by the vertical beam.

Fig. 2 Altitude variation of vertical and horizontal components of air motion estimated from the major spectral component present in Fig. 1*a, c, d* (solid line). Positive vertical speed indicates updraft of air. Horizontal velocity is given in terms of wind speed and direction following the meteorological convention. The beams in the northward and eastward directions that are tilted 15° off from the zenith sample echoes at a range 100–300 m lower in altitude than that of the vertical beam. This altitude difference is taken into consideration when determining three-dimensional velocities by interpolating the vertical speed at the corresponding altitudes with a spline function. Comparisons are made with horizontal winds measured by rawinsondes launched from Shionomisaki, 150 km to the south of

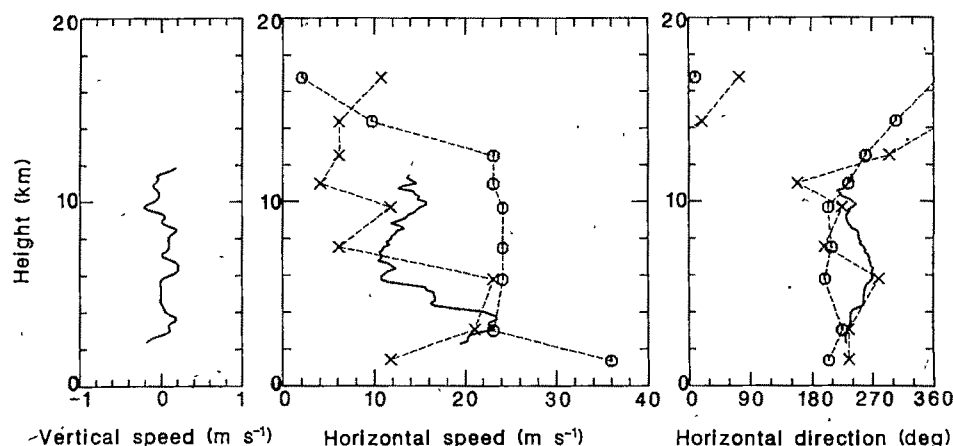
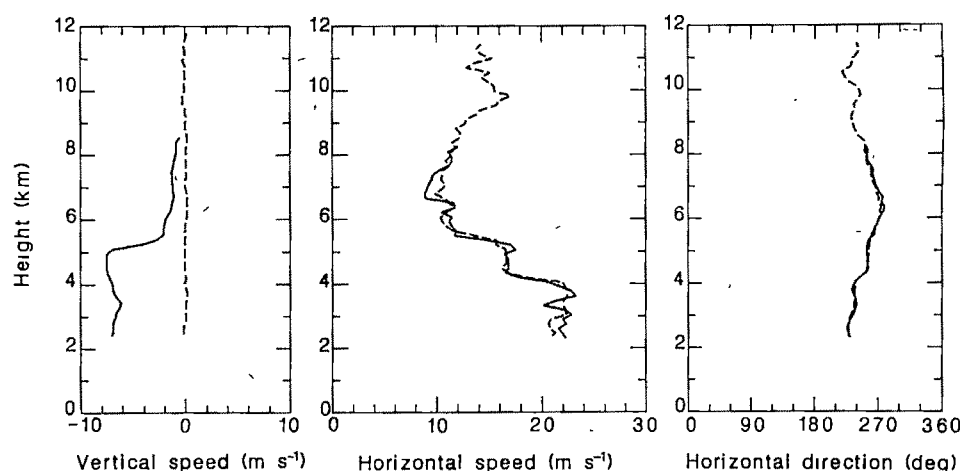


Fig. 3 Mean vertical (fall) speed and horizontal velocity of precipitation particles plotted against altitude estimated from the minor spectral component in Fig. 1a, c, d. Positive vertical speed indicates updraft. The ambient atmospheric wind velocity shown in Fig. 2 is also given by dashed lines for comparison. Precipitation particle motion is not given above 8.69 km, because the spectral component due to precipitation particles merges with that of ambient air origin above this altitude.



Below 5 km, the vertical speed of the minor spectral component is $\sim 7 \text{ m s}^{-1}$, whereas it is $< 2 \text{ m s}^{-1}$ above 6 km (Fig. 3). This change in the altitude range of 5–6 km is quite certain, because the minor spectral component clearly separates from the major one $\approx 8.5 \text{ km}$. It merges with the major component $\approx 9 \text{ km}$.

During periods when rainfall was perceived at the surface level, the melting layer was detected at 5.4 km by a dual frequency (C/Ku band) microwave radar located next to the MU radar¹⁰. Both spectral components of the 46.5-MHz radar echo are also enhanced at this altitude; the enhancement of CAT echo presumably results from a signature in the vertical temperature gradient in the melting layer.

The spectral width of the minor spectral component varies by more than three times near 5–6 km, ranging from 0.8 m s^{-1} above and 2.7 m s^{-1} below the melting layer. It is roughly constant elsewhere.

The above features concerned with vertical speed and spectral width are consistent with those of precipitation particles observed with meteorological Doppler radars^{1,11}. This would indicate that the minor spectral component of the MU radar echo originates from precipitation particles, that is, snowflakes above and raindrops below the melting layer. Note that, by using Doppler velocity spectra obtained by the vertical beam of the MU radar, drop-size distribution functions can be directly determined, obviating the need to make assumptions concerning vertical air motion. By and large, this type of direct measurement is not feasible using conventional meteorological radars.

Mean horizontal speed of precipitation particles is inferred in the same way as that of air (Fig. 3). The precipitation particles of the weak stratiform rain in the present example generally follow the ambient air motion. However, a detailed investigation of the differential motion would certainly be of interest.

In the present observation of a weak stratiform rain, vertical beam and two off-vertical beams with 15° off-zenith angles were used. This assumes that the wind field is uniform over the area containing the three beams, a region $\sim 2 \text{ km}$ at an altitude of 5 km. The assumption of wind field uniformity is necessary in monostatic wind vector measurements. However, it may be a source of error in convective storm environments. Depending on spatial scales of meteorological phenomena observed, a combination of three (or more) beams can be arbitrarily selected from the 1,657 directions within 30° off-zenith angle to which the MU radar can steer its beam. To provide significant three-dimensional motion, minimum beam separation can be reduced to beam width, or 350 m at an altitude of 5 km. This spatial resolution is sufficient to enable the observation of precipitating systems of a moderate size and larger.

Although the MU radar is capable of observing a substantial part of the deep and strong updrafts associated with frontal passages or convective storms which generally extend above an altitude of 10 km, it cannot provide the information concerning activities in the planetary boundary layer. Therefore, simul-

and/or other conventional instruments (such as the rawinsonde) will have an important supporting role in future investigations of the tropospheric meteorological phenomena which employ the MU radar.

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Indirect electrochemical degradation and dissolution of coal by means of persulphate

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The idea of electrochemical coal conversion has recently been revived^{1–7}. The mechanism of anodic coal oxidation has been shown to be indirect and that the electron transfer is mediated by $\text{Fe}^{3+}/\text{Fe}^{2+}$ leached out of the coal. Although the application of various mediator systems has been discussed in detail, all these systems suffer from either comparatively low reaction rates or high CO_2 current efficiencies. Caro's acid (H_2SO_5), obtained as a hydrolysis product during anodic persulphate formation is a powerful redox mediator system for the degradation of the macromolecular coal structure in mild conditions, providing a nearly complete dissolution of coal in sulphuric acid. We propose here that the high current efficiencies accompanied by low CO_2 evolution might open a route of potential interest for the electrochemical production of aromatic carboxylic acids from coal.

This redox mediator system has not previously been described, although there seems to be a similarity between Caro's acid and TFA (trifluoroperacetic acid) the application of which is described elsewhere^{8,9}. However, there are two significant differences. First, TFA destroys aromatic structures to such an extent that Deno proposed TFA-oxidation as a suitable tool for estimating the aliphatic carbon content in coal. The second

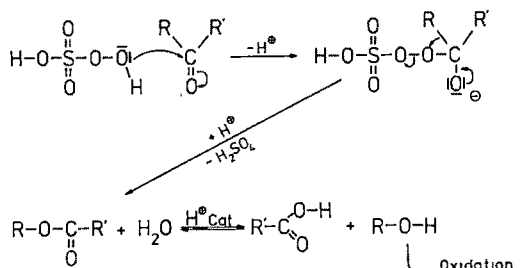
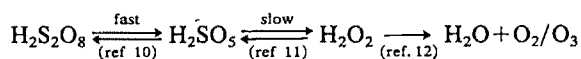


Fig. 1 Mechanism of oxidative coal cleavage by Baeyer-Villiger/Oxidation and consecutive saponification. R and R' represent the rest of the coal structure.

trifluoroacetic acid in conditions which are suitable for industrial application.

The special chemical and electrochemical features of the oxidant persulphuric acid have been described as follows: (1) Despite the high redox potential ($E^\circ = 2.05$ V), persulphate itself reacts, in general, very slowly. (2) In strongly acid solution, $H_2S_2O_8$ is hydrolysed depending on the reaction conditions in accordance with the following scheme



(3) The efficiency of anodic $S_2O_8^{2-}$ formation depends on the concentration of H_2SO_5 since the latter can depolarize the anode with formation of O_2 and H_2SO_4 (ref. 13). (4) Caro's acid (H_2SO_5) is a very strong and highly reactive redox mediator and oxidizes carbonyl groups giving rise to esters and lactones. The mechanism of this 'Baeyer-Villiger-oxidation' reaction, is given in Fig. 1. (All examples of the Baeyer-Villiger-oxidation up to 1953 are reviewed in ref. 14. Many of the compounds mentioned may serve as model substances for coal.)

Coal has a macromolecular structure containing condensed aromatic systems linked together by aliphatic bridges, the most abundant of which are methylene bridges. These units may be easily oxidized to carbonyl groups in the presence of $S_2O_8^{2-}$, SO_3^{2-} or H_2O_2 . A consecutive Baeyer-Villiger-reaction would result in the formation of esters which are supposed to undergo saponification in the strong acid media. By this sequence of reactions, C-C bonds connecting aromatic units in coal should be cleaved giving rise to the formation of low-molecular aromatic carboxylic acids. Because Caro's acid is consumed thereby, the current efficiency for anodic persulphate formation should be improved in the presence of coal according to point (3) above. These assumptions are confirmed by the experimental results which have been obtained in the system $S_2O_8^{2-}/H_2SO_4$ both with and without electrochemical regeneration of the oxidant.

If a given amount of coal (Westerholt-Kohle, West Germany; 2.4 g; <500 μm) is suspended in 300 cm^3 91 wt% sulphuric acid and stirred at 37 °C for 1 h no significant degradation of the coal takes place. If this experiment is repeated in the presence of $Na_2S_2O_8$, the electrolyte quickly turns brown and the coal quantity recoverable by filtration is decreased. This is shown in Fig. 2 for various amounts of $Na_2S_2O_8$ consecutively added to the suspension (curve a). The gas evolved during the reaction (curve c) is mainly O_2 formed by a parallel decomposition of Caro's acid. This gas contains only small amounts of CO_2 . The

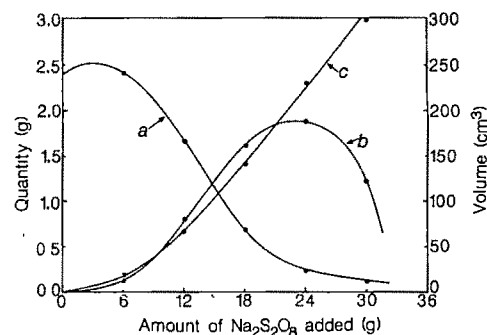
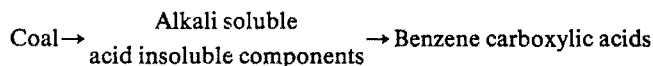


Fig. 2 Coal degradation as function of $Na_2S_2O_8$ consumption. a, Coal recoverable by filtration; b, precipitable acids; c, gas evolution.

losses of $Na_2S_2O_8$ due to gas formation were found to be <25% even at high degrees of coal oxidation.

The detached electrolytes from the experiments given in Fig. 2 were diluted with water in the ratio of 1:6. A deep-brown precipitate of carboxylic acids was collected, the amount of which is shown by curve b. The decrease of the precipitate yield at $Na_2S_2O_8$ consumption >24 g has to be attributed to consecutive oxidation of these intermediates. Thereby, a further breakdown can be achieved which finally results in the formation of low-molecular acids which are soluble and cannot be precipitated by dilution. Oxidative coal degradation follows the common sequence¹⁵:



But it is also known¹⁶⁻¹⁸ that the first step in this sequence is rate determining and cannot be obtained by mild oxidants such as H_2O_2 . Therefore, we were mainly interested in the formation of these intermediates, sometimes termed 'regenerated humic acids', because their consecutive conversion do not need the high redox potential of the persulphate system. In contrast with other oxidants, such as MnO_4^- , O_3 or HNO_3 a complete dissolution of coal can be achieved within a few hours using Caro's acid. Table 1 shows that there is a drastic change in the O/C-ratio between the intermediate acids and the native coal whereas, in spite of the reaction medium used, the sulphur content remains unchanged.

Infrared and nuclear magnetic resonance (NMR) spectral analysis data show that the aromatic basic structure of the coal is preserved within these acids. About 4-6 carboxylic groups per molecular unit were found whereas only a few aliphatic protons can be detected.

The experiments reported here were conducted at 37 °C with a H_2SO_4 concentration of 91 wt%. These conditions were found to be optimum with respect to reaction rate and $Na_2S_2O_8$ losses through gas evolution. At elevated temperatures in sulphuric acid of low concentration, the reaction mixture is decomposed to yield O_2 and O_3 with no significant dissolution of coal, whereas at higher H_2SO_4 concentrations and higher temperatures (~100 °C) parallel SO_2 evolution was observed.

Kinetic investigations have shown that the reaction rate is strongly influenced by H_2SO_4 concentration and that in 3 M sulphuric acid no conversion of coal can be achieved. The same trend was found in $HClO_4$. This fact is in accordance with the proposed reaction mechanism in which the real oxidizing agent is not the persulphate itself but Caro's acid the equilibrium concentration of which depends on the proton concentration¹¹.

Investigations^{13,19} have shown that a high current efficiency for electrochemical formation of persulphate can only be achieved in the following conditions: high current density of ~0.5-1 A cm^{-2} ; low temperature in the range of ~10-15 °C; high SO_4^{2-} concentration; in conditions where the accumulation of H_2SO_5 in the electrolyte is avoided.

This situation is shown in Fig. 3a, where a current efficiency

Table 1 Ultimate analysis of the precipitable acids

	C	H	O	N	S	O/C	Average relative molecular mass
(g)	100	80.9	8.6	1.70	0.80	0.09	5,000-10,000
6	100	76.2	38.2	2.22	1.04	0.38	652
12	100	76.3	44.0	2.17	0.83	0.44	688
18	100	74.2	50.6	2.20	1.04	0.51	732
24	100	79.3	54.9	2.01	0.93	0.55	701
30	100	72.2	52.8	2.27	0.86	0.53	811

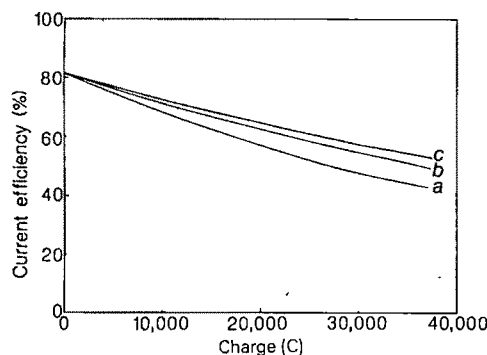


Fig. 3 Differential current efficiency of anodic persulphate formation with and without coal: a, no coal; b, with 7.36 g coal; c, with 14.72 g coal. $\text{H}_2\text{SO}_4 = 65\%$; temperature 10°C ; current 0.75 A ; area of electrode 1 cm^2 ; volume of reactor, 460 cm^3 .

decreases with increasing accumulation of Caro's acid in the electrolyte. Three main effects are observed when the same experiment is repeated in the presence of coal.

First, neither the ash content nor the coal itself or its oxidation products reduce the current efficiency of the electrolysis. Second, no significant differences with respect to coal dissolution is found if electrochemically-regenerated persulphate were used. This can be shown by a further experiment. A total charge of $44,500\text{ C}$ has been applied corresponding to the formation of $0.134\text{ mol S}_2\text{O}_8^{2-}$ with an integral current efficiency of 58.1% determined by measuring the amount of oxygen produced. This electrochemically-formed $\text{S}_2\text{O}_8^{2-}$ dissolved 0.881 g of the 8.16 g of coal used in this experiment, corresponding to a relative coal dissolution of 10.8% . This means a relative persulphate consumption of $0.152\text{ mol S}_2\text{O}_8^{2-}\text{ g}^{-1}$ dissolved coal. For the chemical coal dissolution experiment (Fig. 2) at the same relative degree of coal conversion a persulphate consumption of $0.150\text{ mol S}_2\text{O}_8^{2-}\text{ g}^{-1}$ dissolved coal can be inferred which is in close agreement with the electrochemical experiment. Third, the decrease of current efficiency with time is not as steep as in pure H_2SO_4 , and depends on coal concentration.

The third observations were predicted on the basis of the reaction scheme outlined above, whereas the other observations could not be taken for granted.

The fact that there is still a decrease in current efficiency with increasing charge consumption, even in the presence of coal, has to be attributed to the low reaction rate caused by the low temperature needed in the electrochemical cell. Therefore, an outer cell process seems to be more appropriate where a coal slurry of high concentration is passing the electrochemical cell at low temperature levels and is oxidized in a chemical reactor at about $30\text{--}35^\circ\text{C}$. From the data given in Fig. 3 and from present investigations, a high constant current efficiency can be realized²⁰.

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Noble gases from oceanic island basalts do not require an undepleted mantle source

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Several lines of evidence based on relative rare earth abundances and on strontium, lead and neodymium isotopic geochemistry have led to the suggestion that the Earth's mantle may be divided roughly into a region depleted in volatile components, feeding the mid-ocean ridge basalts (MORB's), and a less depleted ('undepleted') region which feeds hotspots and thus oceanic island basalts (OIB's) such as Iceland and Hawaii. Models of mantle degassing and atmospheric evolution have been based on differences in argon and xenon isotope ratios measured in samples which supposedly trap rare gases from these two reservoirs¹⁻⁷. However, the ultra-mafic Ar and Xe that were first suggested as samples of the undepleted mantle were subject to air contamination and diffusion loss, so that the experimental results are not definitive⁸. New heavy rare gas data on volcanic glasses from Hawaii and Iceland are more directly comparable with the MORB glass data, and supposedly show the isotope pattern expected from the undepleted mantle^{2,5}. As I report here, however, absolute abundances and elemental ratios corrected for mass fractionation effects indicate that partial loss of the depleted mantle component plus addition of an atmospheric Ar and Xe component provide a better rationale for the isotopic differences in these heavy gases than does the postulated undepleted mantle component.

Table 1a summarizes the evidence cited. The trapped component seen in all oceanic glasses (Table 1a) has a $^3\text{He}/^4\text{He}$ ratio higher than atmospheric; as the source is shielded from any extraterrestrial influx, this must indicate a primordial component; the argument continues that the higher ratio seen in the supposedly undepleted glasses then indicates a more primitive source, that is, a more undepleted source.

This difference in ratios between the two mantle components could not have been quantitatively predicted *a priori*, because the undepleted mantle must contain not only higher abundances of the primordial gas (with respect to the depleted mantle), but also more uranium and therefore more radiogenic ^4He . The balance between the primordial ^3He and ^4He and the radiogenic ^4He is impossible to calculate because we do not know the fractionation factor between He and U in mantle processes, the fraction of the original mantle depleted in both the depleted and undepleted (actually merely less depleted) regions, or the time interval since the depletion process or processes occurred.

The $^{40}\text{Ar}/^{36}\text{Ar}$ ratio in the atmosphere is 295.5. The high ratio seen in the depleted samples results from potassium decay in the mantle and therefore is a firm fingerprint for a non-atmospheric origin. The nearly-atmospheric ratio in the undepleted samples has been interpreted as resulting from K-decay in a mantle region where the $\text{K}/^{36}\text{Ar}$ ratio is similar to that which has fed the atmosphere, and is much lower than that of the depleted mantle region because of a much higher primordial ^{36}Ar content⁷. However, the nearly-atmospheric $^{40}\text{Ar}/^{36}\text{Ar}$ ratio seen in the undepleted samples (Table 1a) could also be due to incomplete trapping of mantle Ar with unknown (perhaps depleted-type) isotopic ratios plus atmospheric contamination.

The higher than atmospheric $^{129}\text{Xe}/^{130}\text{Xe}$ ratios reported for (depleted) MORB glasses were interpreted as caused by the radioactive decay of ^{129}I after a significant fraction of the primordial Xe had been removed, implying that the depletion of the mantle took place early in the history of the Earth^{4,5}. This interpretation has been contested on various grounds (ref. 9 and R. Hart, personal communication), however, and conflicts with previous data from Hawaiian ultramafic nodules, where a slight anomaly was found in both the supposedly undepleted and

Table 1 Summary of rare gas ratios (a) and abundances (b) in oceanic glasses

Geographical location and type	Postulated mantle source	Ref.	a, Rare gas ratios			b, Rare gas abundances (cm ³ g ⁻¹ , STP)					
			³ He/ ⁴ He (×10 ⁻⁶)	⁴⁰ Ar/ ³⁶ Ar	¹²⁹ Xe/ ¹³⁰ Xe	³ He (×10 ⁻¹²)	⁴ He (×10 ⁻⁸)	³⁶ Ar (×10 ⁻¹⁰)	⁴⁰ Ar (×10 ⁻⁸)	¹³⁰ Xe (×10 ⁻¹⁴)	¹³⁶ Xe (×10 ⁻¹⁴)
MORB glasses	Depleted	5,12,15-18	12±2	500-25,000	648-690	35-450	1,400±1,000	0.3-7	150±100	6-30	0-2
Hawaiian glasses	Undepleted	2,5	17-50	350±50	648	4-40	20-170	1.5-12	0.3-35	4-30	0-1
Iceland											
Atmosphere			1.15	295.5	648						

depleted samples^{6,10,11}. The atmospheric Xe ratios in the OIBs may be evidence of a greater primordial/radiogenic ratio or, alternatively, of a greater atmospheric component.

I propose two arguments to the effect that the observed differences between the two groups of samples result from diffusion loss of mantle gases from and incorporation of atmospheric-type rare gases into the undepleted mantle samples, masking any possible Ar and Xe mantle signatures. First, consider the absolute abundance data for these samples shown in Table 1b. The undepleted mantle should have higher concentrations of all the rare gases than has the depleted system, yet the depleted samples show higher abundances of ³He, ⁴He, ⁴⁰Ar, and fissionogenic ¹³⁶Xe (¹³⁶Xe_f). (A very few MORBs have lower trapped rare gas abundances; the vast majority lie within the limits shown.) If the OIBs really represent undepleted mantle, it follows that they trapped the mantle gases less efficiently than did most MORBs. They were emplaced under shallower water depths, which may certainly explain the observed abundances; nevertheless, without arguing for any particular mechanism, the fact of lesser trapping efficiencies for the OIBs is inescapable if they are to be thought of as arising from the undepleted mantle. And yet these undepleted samples have generally higher abundances of ³⁶Ar, which is precisely the isotope most affected by atmospheric contamination. Thus, the absolute abundances indicate both incomplete trapping and atmospheric contamination, rather than support for the depleted/undepleted mantle concept.

This interpretation is supported by the second argument, which relies on elemental abundance ratios. Such values are notoriously unreliable as model foundations because they are easily influenced by mass fractionating mechanisms; however, some of the data sets have a built-in mass fractionation monitor, the ⁴He/¹³⁶Xe_f ratio¹². ⁴He is the radiogenic daughter of U and Th and their daughters, while ¹³⁶Xe is the fissionogenic daughter of ²³⁸U. (Allègre *et al.*⁵ erroneously claim ²⁴⁴Pu as the fissionogenic nuclide parent; see ref. 9 for a clarification of this point.) The ratio is constant over long periods of geological time, and other evidence indicates that accumulation of the radiogenic rare gases in these samples has indeed proceeded for time periods of the order of hundreds of millions of years⁹. Possible variations in the Th/U ratio introduce only a small variation. Thus, if the ratio found in a sample which has trapped mantle gases is higher than the production ratio of 4.2×10^8 (±10%) (see ref. 12), it reveals the operation of positive mass fractionation (enrichment of the lighter gas), presumably during the mantle/crust emplacement process, while values lower than the production ratio indicate loss of the lighter gas (presumably through diffusion during or after eruption). If this radiogenic/fissionogenic ratio in a particular sample has been thus altered by mass fractionation, the comparable primordial ratio ³He/¹³⁰Xe must necessarily have been identically altered. Therefore, if a suite of samples has trapped rare gases from mantle source regions of identical He/Xe ratios, the measured ⁴He/¹³⁶Xe_f ratios will be proportional to the measured ³He/¹³⁰Xe ratios, regardless of whether any mass fractionation process has been operating on them:

$${}^3\text{He}/{}^{130}\text{Xe} = k_1 ({}^4\text{He}/{}^{136}\text{Xe}_f) \quad (1)$$

where k_1 is a constant. This is borne out by the MORB data of Table 2 (which includes all data sets for which the Xe isotope data are measured sufficiently precisely to allow an estimate of the fissionogenic component of ¹³⁶Xe): with a variation in

⁴He/¹³⁶Xe_f of a factor of 25 and more than an order of magnitude in absolute He and Xe abundances, k_1 remains constant to within ±50%. The surprise is that k_1 for the undepleted samples of Table 1b is identical. (The value for KK-29-10 is only an upper limit because only an upper limit to the ¹³⁶Xe_f can be calculated from the data.) This requires explanation. The ratio of trapped, mass-fractionated gases found in MORBs or elsewhere is related to that of the mantle source region by

$$({}^i\text{He}/{}^j\text{Xe})_{T,\alpha} = k({}^i\text{He}/{}^j\text{Xe})_\alpha \quad (2)$$

where i and j are mass numbers, k is a constant related to the i - j mass difference, α identifies the source region and T,α indicates the trapped component arising from source region α . Then, for a sample trapping rare gases from an undepleted source region ($\alpha = 0$),

$$({}^3\text{He}/{}^{130}\text{Xe})_{T,0} = k({}^3\text{He}/{}^{130}\text{Xe})_0 \quad (3)$$

$$({}^4\text{He}/{}^{136}\text{Xe}_f)_{T,0} = k({}^4\text{He}/{}^{136}\text{Xe}_f)_0 \quad (4)$$

leading to equation (1) where $k_1 = ({}^3\text{He}/{}^{130}\text{Xe})_0 / ({}^4\text{He}/{}^{136}\text{Xe}_f)_0$. The depleted mantle source region of the MORB will have lost more He than Xe in the depletion process (as is evidenced by MORBs showing ⁴He/¹³⁶Xe_f ratios higher than the production ratio), but the $({}^4\text{He}/{}^{136}\text{Xe}_f)_0$ ratio will soon be re-established through continued radiogenic production; the $({}^3\text{He}/{}^{130}\text{Xe})_0$ ratio, on the other hand, is permanently reduced. Therefore

$$({}^3\text{He}/{}^{130}\text{Xe})_{T,D} = k_1' ({}^4\text{He}/{}^{136}\text{Xe}_f)_{T,D} \quad (5)$$

where D indicates the depleted mantle, and

$$\begin{aligned} k_1' &= ({}^3\text{He}/{}^{130}\text{Xe})_D / ({}^4\text{He}/{}^{136}\text{Xe}_f)_D \\ &= ({}^3\text{He}/{}^{130}\text{Xe})_D / ({}^4\text{He}/{}^{136}\text{Xe}_f)_0 \\ &= k_1 ({}^3\text{He}/{}^{130}\text{Xe})_D / ({}^3\text{He}/{}^{130}\text{Xe})_0 \neq k_1 \end{aligned} \quad (6)$$

The ratio $({}^3\text{He}/{}^{130}\text{Xe})_D / ({}^3\text{He}/{}^{130}\text{Xe})_0$ is not unity because of mass fractionation during the depletion process, and therefore $k_1' \neq k_1$. The value of the ratio can be estimated from the data shown here and in previous work. The reasoning is as follows.

Table 2 Rare gas elemental fractionation ratios

Sample	Ref.	$k_1 \times 10^{-5}$	$k_2 \times 10^{-5}$
'Undepleted' mantle			
Loihi Seamount			
KK-24-7	5	0.1	3.28
KK-29-10	5	<0.2	<7.0
Hualalai			
KK-9-14	5	0.13	1.28
MORB 'Depleted' mantle			
ATI 96-18-1	5	0.23	0.06
MD 23 Dr 04	5	0.073	0.04
ALV 981 R23	5	0.25	0.04
ALV 981 R26	5	0.076	0.04
ALV 892-2	5	0.084	0.098-0.21
AT-72	This work	0.090	0.065
AT-72	This work	0.083	0.060
AT-88	12	0.080	—
Average undepleted		0.12±0.02	2.30±1.40
Average depleted		0.13±0.07	0.06±0.02

Either the argon in the OIBs contains an atmospheric component or it does not. If it does, its isotopic composition is not representative of the undepleted mantle; it is only a lower limit to that composition, the exact value being dependent on the relative amounts of trapped mantle and atmospheric argon. If the argon in the OIBs contains no atmospheric component, we may use the measured argon concentrations in OIB and MORB samples as an estimate of the relative degassing that has taken place between the depleted and undepleted mantle, and therefore of the mass fractionation that must have occurred.

^{36}Ar in MORBs has been shown to be a mix of trapped mantle argon and atmospheric contamination^{9,13,14}; limits of trapped mantle ^{36}Ar are $\leq 1.5 \times 10^{-10} \text{ cm}^3 \text{ g}^{-1}$, STP. The highest ^{36}Ar content of OIBs is 12×10^{-10} (Table 1b). We assume for the moment that this represents argon trapped from the undepleted mantle rather than atmospheric contamination; it is a lower limit (compared with the MORB data), as it is clear from the He data that the OIBs have retained less of their mantle-inherited rare gases than have the MORBs. Then the basalts from the depleted mantle have trapped less than 10% of the argon trapped in basalts from the undepleted mantle, although their trapping efficiency is greater; the depletion factor must therefore be greater than an order of magnitude. Given that the mass fractionation occurring during the depletion is proportional to the square roots of the masses, it follows that the ratio $(^{3}\text{He}/^{130}\text{Xe})_{\text{D}}/(^{3}\text{He}/^{130}\text{Xe})_0$ remaining in the two mantles today must be less than 0.017 and therefore $k_1 \leq 0.017 k_i$. However, the data of Table 1b cannot be reconciled with this result; the single value of k_i for both MORBs and OIBs flatly contradicts the expectation based on the concept of depleted and undepleted mantle regions outlined above. I suggest that the large enrichment of k_1 actually observed in the supposedly undepleted samples is a result of the $(^{3}\text{He}/^{130}\text{Xe})_0$ ratio in these samples being lessened by both loss of mantle ^3He and addition of atmospheric ^{130}Xe . This addition of atmospheric Xe is also responsible for the roughly atmospheric $^{129}\text{Xe}/^{130}\text{Xe}$ ratios in these samples.

The analysis can be extended to the Ar/Xe ratio:

$$^{36}\text{Ar}/^{130}\text{Xe} = k_2(^4\text{He}/^{136}\text{Xe}_t) \quad (7)$$

This time a distinct difference is seen between the two groups of samples (Table 2); the constant k_2 is much greater for the undepleted group, indicating either too much ^{36}Ar or too little ^{130}Xe . Since k_1 is identical for both groups, the simplest conclusion is that the samples interpreted as undepleted have an excess of ^{36}Ar . This excess of ^{36}Ar is the outstanding characteristic of samples which have undergone atmospheric contamination, as our atmosphere has a low $^3\text{He}/^{36}\text{Ar}$ ratio and a high $^{36}\text{Ar}/^{130}\text{Xe}$ ratio relative to the planetary primordial component.

The mode of incorporation of the atmospheric gases is unclear. The temperature release patterns of ref. 5 suggest that it is actually dissolved in the glass. If this is so, it may represent an atmospheric component injected into the magma source region rather than what is generally called 'atmospheric contamination'—surface adsorption following emplacement of the magma on the Earth's surface.

To conclude, the rare gas isotope ratio patterns of Table 1a, which have been offered as evidence of gases trapped from the undepleted mantle, are not convincing; two of the three (the $^{40}\text{Ar}/^{36}\text{Ar}$ and $^{129}\text{Xe}/^{130}\text{Xe}$ ratios) could equally well be explained by an atmospheric rather than an undepleted component. The data of Tables 1b and 2 cannot be explained by an undepleted mantle component but are perfectly explicable in terms of incomplete trapping and atmospheric admixture. The one data set that seems to indicate the existence of the undepleted mantle, the $^3\text{He}/^4\text{He}$ ratio, is also the one that would be least affected by the presence of an atmospheric component. It follows that modelling of mantle evolutionary histories and atmospheric formation which are based on Ar and Xe isotope differences between the depleted and undepleted mantle are not supported by such data as are now available.

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Thorium-232 in the eastern Caribbean Sea

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The radiogenic Th isotopes (^{234}Th , ^{230}Th and ^{228}Th) are produced *in situ* in the ocean from their dissolved progenitors (^{238}U , ^{234}U and ^{228}Ra , respectively). These parent-daughter pairs have extensively been used to study the rate and mechanism of chemical scavenging, a process that is believed to be important in controlling the distributions and concentrations of many trace metals, such as Be, Al, Cr, Mn, Fe, Co, Cu, Pb and the rare earths^{1–12}. However, it is difficult to make useful direct comparisons between the distributions of the radiogenic Th tracers and those of trace metals, because their input pathways to the ocean are very different. To provide a link, one needs to determine the water-column distribution of the dominant isotope ^{232}Th , which is non-radiogenic and is thus delivered to the ocean by the same fluvial and aeolian pathways as most trace metals. However, with the exception of the near-bottom measurements recently reported by Nozaki and Horibe¹³, little is known of the concentration level of ^{232}Th or its distribution pattern in the ocean. We report here the first profile showing the complete distribution of ^{232}Th in an oceanic water column from the Venezuelan Basin. The data were obtained by a sensitive neutron activation analysis method newly developed for this project. The profile shows relatively high concentrations in the upper 200 m with a maximum of $87 \text{ d.p.m.}/10^6 \text{ kg}$ near the base of the mixed layer. Average concentrations in deeper water ($2.7 \text{ d.p.m.}/10^6 \text{ kg}$) are uniform and atypically low compared with open-ocean deep waters, perhaps because of unusually rapid scavenging in the restricted basin. The source of the ^{232}Th in the surface waters is not known, but rivers appear to be more likely candidates than the atmosphere.

The samples were collected at $14^\circ 31' \text{ N}$, $66^\circ 8' \text{ W}$ during R/V *Knorr* Cruise 99–2 in November 1982. The seawater was filtered ($1 \mu\text{m}$ pore-size Nuclepore filters) and drained into 10-l acid-cleaned jerryjugs directly from the PVC Niskin samplers. Following collection, 12.5 cm^3 double quartz-distilled 8M HCl was added to each sample to bring the pH to ~ 2 . A full description of the clean sampling and filtration procedures used at sea is given elsewhere¹⁴.

Analysis in the shore-based laboratory began with additions of purified Fe^{3+} carrier and ^{230}Th spike to the acidified water. After several days for equilibration, ammonium hydroxide (ULTREX) was added to effect $\text{Fe}(\text{OH})_3$ precipitation. The

precipitates were collected by centrifugation and then dissolved in HCl. Separation and purification of Th-isotopes were done by anion and cation exchange and solvent extraction according to the method of C.-A.H.¹⁵ with minor modifications. The final source (in thenoyltrifluoroacetone/benzene) was mounted onto a high-purity (Puratronic) Al foil (2.5 cm diameter, 0.025 mm thickness). The Th recovery, based on ²³⁰Th, was determined with a proportional counter.

The sample foils, together with standard and blank foils, were packed in a polyethylene vial and irradiated with a thermal neutron flux of 5×10^{13} neutron $\text{cm}^{-2} \text{s}^{-1}$ for 6 h at the MIT Nuclear Reactor Laboratory. After removal from the reactor, the samples were allowed to cool for four days before the post-irradiation extraction of ²³³Pa (induced from ²³²Th).

The irradiated Al foils were dissolved in concentrated HCl and spiked with ²³¹Pa. The procedure for Pa (²³¹Pa and ²³³Pa) extraction was the same as that given in ref. 15, and the final source was made on a stainless steel disk. The Pa yield was determined by α -counting ²³¹Pa in a proportional counter. The ²³³Pa was determined by β -counting ²³³Pa in the same proportional counter. The purity of the Pa source was confirmed by the distinct 312-keV γ -radiation observed in a γ -spectrometer and by the decrease of β -activity being consistent with the half-life (27 days) of ²³³Pa. A complete description of the analytical procedures is given elsewhere¹⁶.

In dealing with the extremely low ²³²Th activities in the samples, we were aware of the potential for sample contamination and the importance of blank correction. Four sample blanks (reagents + Al foil) and three foil blanks (Al foil only) were prepared along with the samples and standards. Figure 1 shows, for these blanks, the relationship between the activity of ²³³Pa detected and the recovery of Pa in the post-irradiation chemical procedure. Two straight regression lines, one drawn for the sample blanks (SB1-4) and one for the foil blanks (FB1-3), are shown in Fig. 1. The offset of these two lines (that is, sample blank above foil blank) is a measure of the reagent blank, which turns out to be small compared with the foil blank. The good fit of the lines to the points indicates that ²³²Th on the Puratronic Al foil, although it exists at an easily detectable level, is evenly distributed in the material. The regression line for the sample blanks was used in making blank corrections for the samples and standards.

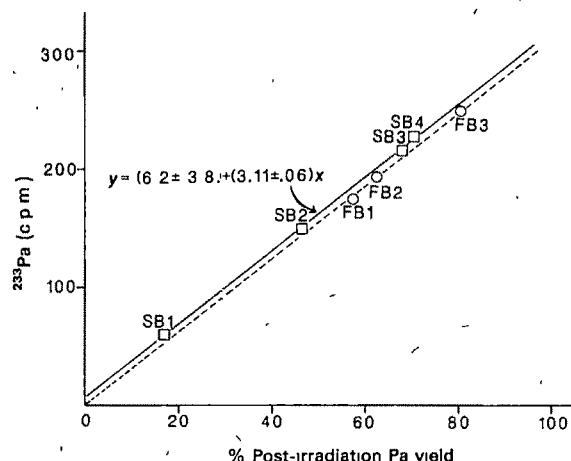


Fig. 1 The ²³³Pa/Pa yield relationship for the sample blanks (□) and Al-foil blanks (○). The slight offset of the two regression lines indicates that the foil blank dominates over the reagent blank.

Four ²³²Th standards, ranging from 0.01 d.p.m. to 2 d.p.m. (disintegrations per minute), were prepared from high purity ThO₂ (Lindsay Code 116, >99.9% purity) and run through the same procedures with the samples. A linear response resulted, as expected (Fig. 2); the regression coefficient is 1.24×10^6 (counts per minute, c.p.m. ²³³Pa/d.p.m. ²³²Th). The activities of ²³²Th in the samples were then calculated using the equation:

$$^{232}\text{Th (d.p.m. kg}^{-1}) = \frac{(\text{c.p.m. } ^{233}\text{Pa})_{\text{measured}} - (\text{c.p.m. } ^{233}\text{Pa})_{\text{blank}}}{(\% \text{ Th yield}/100)(\% \text{ Pa yield}/100) 1.24 \times 10^6 (\text{c.p.m.}/\text{d.p.m. sample wt (kg)})}$$

The results (Table 1) show that the sample blanks (reagents + foil) contribute 25–98% of the signals detected, resulting in uncertainties (by standard propagation of errors) of 2–80% for the final ²³²Th data.

The ²³²Th profile (Fig. 3) shows increasing concentrations with depth down to a maximum at 70 m, which coincides approximately with the base of the mixed layer defined by the temperature and salinity profiles. A weak suggestion of a

Table 1 Data used to calculate ²³²Th concentrations at the Caribbean Sea station (14°31' N, 66°8' W; water depth 4,886 m)

Sample no.*	Water depth (m)	Sample weight (kg)	% Th yield	% Pa yield	c.p.m. ²³³ Pa		²³² Th† (d.p.m./10 ⁶ kg)
					Measured	Corrected†	
601	10	10.6	70 ± 2	63 ± 2	272	70 ± 6	12.1 ± 1.1
602	40	10.8	80 ± 2	70 ± 2	642	418 ± 6	55.7 ± 1.6
603	70	10.8	83 ± 2	82 ± 1	1045	787 ± 3	87.4 ± 2.1
108	99	10.4	69 ± 2	73 ± 2	515	281 ± 6	43.3 ± 1.6
604	150	8.7	79 ± 2	80 ± 2	427	172 ± 6	25.2 ± 0.9
109	198	11.0	92 ± 1	90 ± 1	510	224 ± 3	19.8 ± 0.3
111	397	10.1	94 ± 1	55 ± 1	193	16 ± 3	2.5 ± 0.5
113	597	9.8	85 ± 2	63 ± 2	209	7 ± 6	1.1 ± 0.9
115	895	10.1	81 ± 2	74 ± 1	240	4 ± 3	0.5 ± 0.4
116	1,195	10.8	78 ± 2	59 ± 2	207	17 ± 7	2.8 ± 1.1
117	1,493	10.0	91 ± 1	83 ± 1	285	21 ± 3	2.2 ± 0.3
118	1,789	10.0	85 ± 2	94 ± 1	334	35 ± 4	3.5 ± 0.4
407	2,084	10.8	64 ± 2	92 ± 1	297	5 ± 3	0.6 ± 0.4
409	2,684	11.0	59 ± 2	49 ± 2	174	15 ± 7	3.8 ± 1.8
411	3,283	9.8	63 ± 2	76 ± 2	259	16 ± 7	2.8 ± 1.2
413	3,882	10.6	39 ± 2	79 ± 1	274	22 ± 3	5.4 ± 0.9
415	4,382	9.6	69 ± 2	80 ± 2	269	14 ± 6	2.1 ± 0.9
416	4,582	11.2	83 ± 1	92 ± 1	320	28 ± 3	2.6 ± 0.3
417	4,682	10.4	88 ± 1	63 ± 2	224	22 ± 6	3.1 ± 0.8
418	4,782	10.8	76 ± 1	61 ± 1	227	31 ± 3	5.0 ± 0.5

1 d.p.m. = 0.0167 Bq, 1 d.p.m. ²³²Th = 4.09 μg = 0.0176 μmol

* First digit designates cast number; second and third designate bottle number

† $^{233}\text{Pa}_{\text{corrected}} = ^{233}\text{Pa}_{\text{measured}} - ^{233}\text{Pa}_{\text{blank}}$, where $^{233}\text{Pa}_{\text{blank}}$ is estimated from Fig. 1.

‡ $^{232}\text{Th} = ^{233}\text{Pa}_{\text{corrected}} / [(\% \text{ Pa yield}/100)(\% \text{ Th yield}/100)(1.24)(\text{sample wt.})]$.

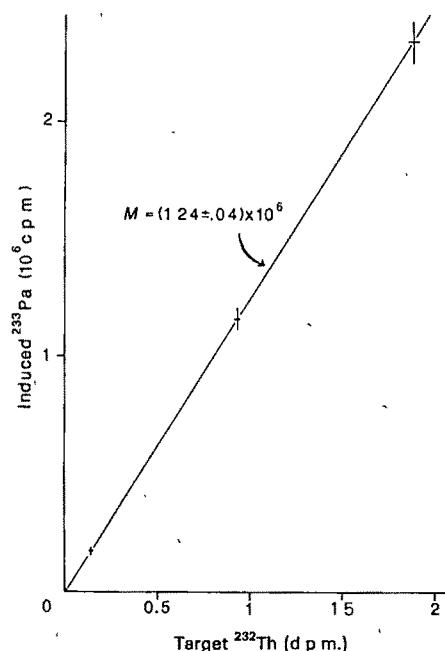


Fig. 2 Calibration line of ^{233}Pa detected (c.p.m.) versus ^{232}Th in the target (d.p.m.).

minimum is found at ~600–900 m. From 1,200 m to the bottom, the water column is very weakly stratified and there is no systematic trend of the ^{232}Th concentration with depth.

Many of the earlier reports of the ^{232}Th concentration in seawater acknowledged large uncertainties caused by the possibility of sample contamination and the large blank corrections that were necessary. Because all of our analyses of deeper samples (>200 m) showed consistently low concentrations (Table 1), we are confident that the much higher values we report for the upper 200 m accurately represent the concentrations in those samples. Our 10-m value is close to the upper limit of 17 d.p.m./ 10^6 kg for surface seawater set by Kaufman¹⁷ and later confirmed by Knauss *et al.*¹⁸. It is also within the range of 10–20 d.p.m./ 10^6 kg recently measured by Moore¹⁹ in surface seawater samples from the eastern Pacific. The one previously reported²⁰ value for surface Caribbean water (160 d.p.m./ 10^6 kg) is almost certainly too high.

The concentrations of ^{232}Th measured in the subsurface maximum are considerably higher than the upper limit for surface seawater given in earlier work. Whether these high concentrations are due to vertical particulate transport of ^{232}Th and its release at the depth of the maximum, or whether they are due to advective transport from a remote source, cannot be decided without additional data. During an earlier occupation of the same station, a ^{210}Pb maximum was found at nearly the same depth²¹.

Our deep-water values are considerably lower than most all others previously reported^{13,18–20,22,23}. The 14 samples from 397 to 4,782 m gave a mean concentration of 2.7 d.p.m./ 10^6 kg and a standard deviation of 1.4 d.p.m./ 10^6 kg. The results were close to our detection limit and could not be determined with very good precision. Improvement of precision at these very low concentrations would require larger samples or a reduction of the Al foil blank. The only value of comparably low magnitude that has been reported is the <2 d.p.m./ 10^6 kg determined by Anderson *et al.*²⁴ on a single sample from the deep North Pacific obtained with a moored adsorber. Recently, Nozaki and Horibe¹³, also using moored adsorbers, reported values ranging from 6.6 to 33.0 d.p.m./ 10^6 kg in the bottom 2,000 m at a 5,800-m station in the western North Pacific. Their values showed a regular decreasing trend with height above the bottom, suggesting a bottom source of ^{232}Th . We have not observed evidence for this in the Caribbean, where the deep water is well mixed.

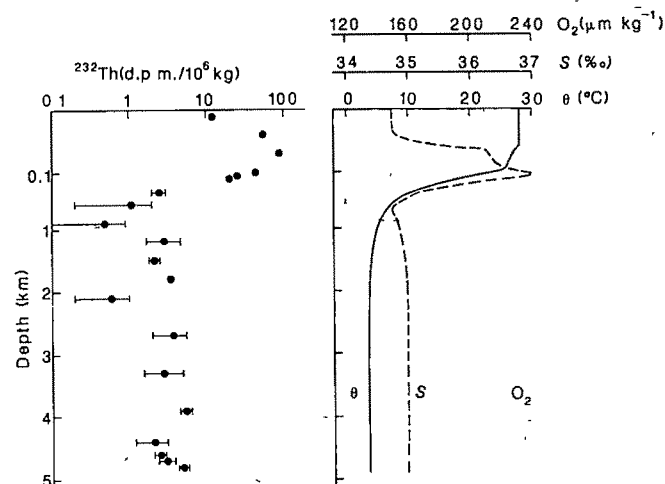


Fig. 3 Water-column ^{232}Th profile at the Caribbean Sea station (14°31' N, 66°8' W). Also shown are the profiles of potential temperature (θ), salinity (S), and dissolved O_2 .

Although some of the earlier reported ^{232}Th data may be erroneously high because of contamination, it would be premature to reject them before further measurements are made. It may well be that ^{232}Th concentrations in the deep Caribbean are anomalously low by comparison with other regions. The overall pattern of the ^{232}Th distribution, with high concentrations near the surface and low concentrations at depth, is strongly suggestive of control by chemical scavenging in the deep water, and there is evidence that scavenging in the Venezuelan Basin is unusually rapid because of the enclosed nature of the basin and the proportionately large effect of uptake at the boundaries. The residence time of ^{210}Pb in the deep Venezuelan Basin was found to be only 20 yr (ref. 25), compared with values of ~50–200 yr more typical of open-ocean areas^{25,26}. Our unpublished ^{230}Th data obtained by large volume, *in situ* samplers, indicate a Th residence time of ~10 yr in the Venezuelan Basin, compared with open-ocean values of ~20–30 yr (refs 23, 27). Intense scavenging could maintain anomalously low concentrations of ^{232}Th .

The ^{232}Th concentration reported here for filtered seawater samples represents only a small fraction of the total. Our unpublished large volume results for the particulate matter gave an average of 22 d.p.m. $^{232}\text{Th}/10^6$ kg for nine samples collected below 200 m at the same station, or about 90% of the total ^{232}Th . This is in contrast to ^{230}Th , which is generated by decay of dissolved ^{234}U in the water column. Only ~30% of the total ^{230}Th occurs in the particulate form.

The new data reported here, showing the relatively high surface concentrations, raise the question of how dissolved ^{232}Th is supplied to the surface ocean—whether by leaching of dust delivered from the atmosphere or by river input. As discussed by Measures *et al.*²⁸, the question is a generally difficult one for trace metals, and with our limited data we can only advance a few tentative statements regarding the magnitude of the supply that is necessary. From our data, the inventory of dissolved ^{232}Th in the upper 200 m is 6.5 d.p.m. m^{-2} , and from studies of ^{228}Th , it is known that the residence time of dissolved Th in the upper ocean averages ~0.7 yr (ref. 29). Thus a supply rate of 9.3 d.p.m. $\text{m}^{-2} \text{yr}^{-1}$ is required to maintain the inventory of ^{232}Th that we observe. Buat-Menard and Chesselet³⁰ estimated the flux of ^{232}Th in the form of atmospheric dust over the tropical North Atlantic to be 2.2 d.p.m. $\text{m}^{-2} \text{yr}^{-1}$. Because only a small fraction of that flux would be leached on contact with surface seawater, this does not appear to be an adequate source. Moore³¹ reported a value of 0.024 d.p.m. $^{232}\text{Th} \text{kg}^{-1}$ in centrifuged Amazon River water. Taking that value and the river flux to the Atlantic of 250 $\text{kg} \text{m}^{-2} \text{yr}^{-1}$ yields a ^{232}Th flux of 6 d.p.m. $\text{m}^{-2} \text{yr}^{-1}$, not much lower than the required supply rate estimated above. Considering that the Caribbean is probably more affected

by river input than the average Atlantic surface water, river input may be an adequate source of ^{232}Th , although nothing is known about the estuarine behaviour of Th.

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Regressive events in the postnatal development of association projections in the visual cortex

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In newborn kittens, neurones in area 17 of the visual cortex projecting to area 18 are distributed in bands of uniform density across the superficial layers (laminae II, III and the upper part of IV) and the deep layers (V and VI). During weeks 2 and 3 postnatal, the cells of origin of this association pathway become mainly restricted to discrete, dense clusters, ~600 μm from centre to centre, in the upper layers, with intervening zones free of association cells^{1,2}, as in the adult cat^{3,4}. We have used retrogradely transported, long-lasting neuronal markers to investigate this developmental refinement of the pattern of cortico-cortical connections. The results, reported here, indicate that axonal retraction plays a significant part in the maturation of the clustered organization of superficial layer neurones projecting to area 18, but that cell death may also be a factor in the elimination of the inappropriate projection from the deep laminae.

Recent research has demonstrated that certain neurones in the mammalian cerebral cortex, early in development, have axonal connections to targets that would be inappropriate in the adult animal. For the long interhemispheric and cortico-spinal pathways, the postnatal elimination of these initially 'exuberant' projections seems to be achieved by axon retraction without death of the cells of origin^{5,6}. Neuronal death certainly occurs in many parts of the developing nervous system⁶, including during the early stages of formation of the cerebral cortex⁷, but it has not yet been shown to be involved in the elimination

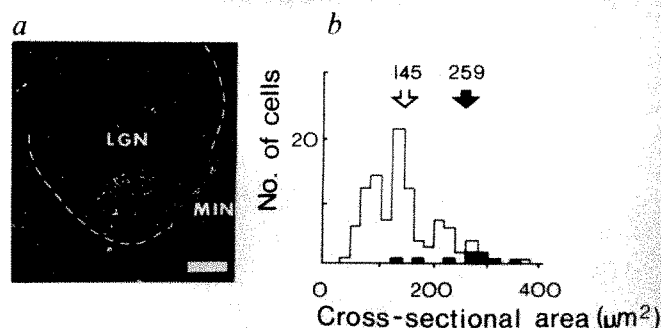


Fig. 1 Analysis of LGN cells retrogradely labelled following an injection of WGA-HRP into area 18 in a 4-day-old kitten. *a*, Dark-field illuminated coronal section through the thalamus, showing labelled cells lying in a central position in the LGN, as well as in the medial interlaminar nucleus (MIN). The lateral and ventral borders of the LGN are indicated by the broken line. Scale bar, 1 mm. *b*, Histogram of cross-sectional areas of a sample of cells from the middle of the region of uptake. Solid blocks represent labelled cells; mean values (in μm^2) are indicated for total (open arrow) and labelled (solid arrow) samples. Injections that produced labelling of relatively large, centrally placed cells in the LGN were considered to be confined to area 18.

of exuberant cortical projections. In the present study we examined the factors involved in development of the relatively short cortico-cortical projections from area 17 to area 18 in kittens.

Kittens were anaesthetized with halothane, fixed in a stereotaxic holder⁸ and axonally transported tracers were injected into area 18 of the visual cortex. Three animals, two aged 2 days and the third aged 2 months, were given a single 500 nl injection of a 2% solution of the fluorescent tracer fast blue (FB) via a pulsatile pressure system through a glass micropipette (tip diameter ~50 μm) into area 18 on the left side of the brain. These kittens then survived for 4 weeks before perfusion with a 4% solution of paraformaldehyde. In one of these animals the initial injection of FB at 2 days was followed at 1 month old, 24 h before perfusion, by a second injection of diamidino yellow (DY; see ref. 9) into area 18 close to the original FB injection site, using identical methods. In a further four kittens (aged 1, 4, 10 and 20 days) area 18 was injected iontophoretically (repetitive positive current pulses of 3 μA for 2 h) with a 10% solution of horseradish peroxidase conjugated with wheat-germ agglutinin (WGA-HRP) dissolved in a solution of Tris(hydroxymethyl)methylamine; these animals were perfused either 8 or 24 h later with 1.25% glutaraldehyde and 1% paraformaldehyde.

Coronal sections 50 μm thick were cut through the visual cortex and lateral geniculate nucleus (LGN) on the side of the injection; material containing WGA-HRP was reacted using tetramethylbenzidine as the chromogen¹⁰ and counterstained with neutral red. Sections were examined using light or fluorescence microscopy and drawings were made of the positions of all labelled, or, where appropriate, double-labelled cells, within the cortex and LGN. For each animal a series of sections through the visual cortical areas was counterstained with cresyl violet and another was reacted to reveal cytochrome oxidase activity¹¹. We made high-power drawings of the outlines of all labelled cells (in which the nucleus and nucleolus were visible) within an area in the centre of retrograde labelling in the LGN, and the cross-sectional areas of these cells were calculated using a graphics tablet linked to a computer. The sizes of these labelled cells were compared with those of a sample of intermingled unlabelled Nissl-stained LGN neurones from within the same area.

The injection sites of FB and DY in area 18 were all ~1 mm wide but covered the entire depth of the cortex so as to expose axon terminals in all laminae to the tracer. Haloes of staining around the deposits of WGA-HRP were slightly larger and more variable in size (up to 2 mm wide), although the effective uptake sites of WGA-HRP¹⁰, estimated from the pattern of label in the

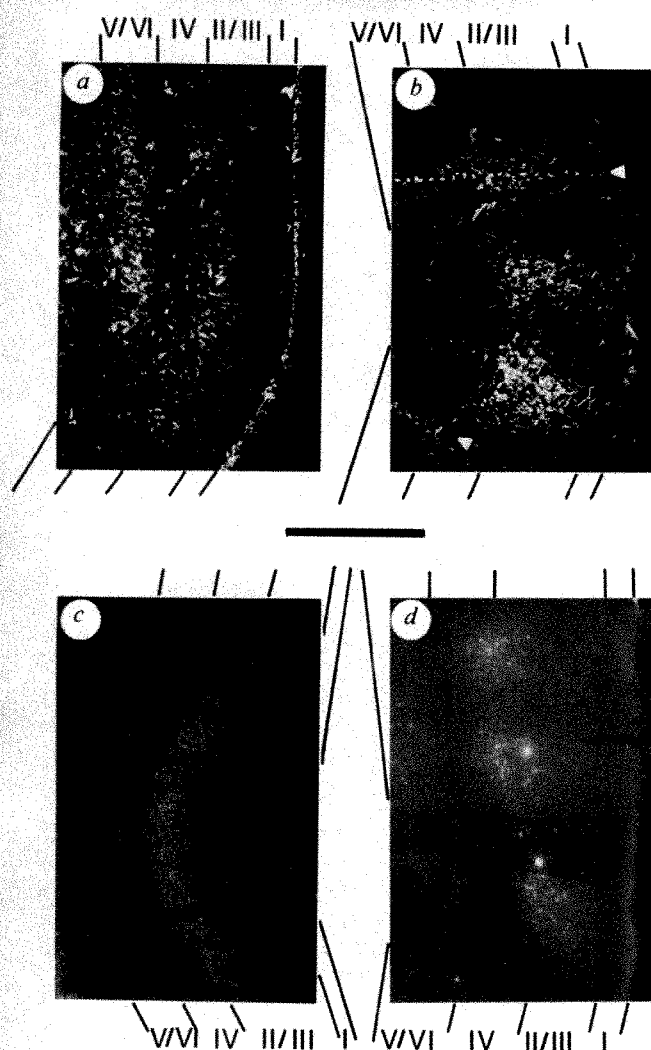


Fig. 2 Photomicrographs of area 17, showing labelling of neurones with FB or WGA-HRP after injections into area 18. *a*, At 8 h after injection of a 4-day-old kitten with WGA-HRP, showing labelled cells lying in continuous bands in the superficial and deep layers; *b*, 24 h after injection of a 20-day-old kitten with WGA-HRP in which labelled cells lie in patches mainly in the upper layers; *c*, 4 weeks after injection of a 2-day-old kitten with FB, showing persistent continuous bands of labelled cells that are much denser in superficial than in deep layers; *d*, 4 weeks after injection of a 2-month-old kitten with FB, showing clusters of labelled cells mainly in the superficial layers, with few in the deep layers. *a*, *b*, Dark-field-illuminated; *c*, *d*, fluorescence photomicrographs. Artefactual label is seen along the pial surface in *a* and *b*, and white arrowheads in *b* indicate radial blood vessels. Scale bar, 1 mm.

LGN, were probably less than 2 mm across. The positions of injection sites relative to the borders of area 18 were determined not only by direct examination of the cytoarchitectonics¹² and cytochrome oxidase activity¹³ of the surrounding cortex, but also by inspection of the distribution and size of labelled cells in the LGN. In all animals the group of labelled cells lay in the centre of the LGN (Fig. 1*a*) in the coronal plane, not extending to either the medial or lateral edges of the nucleus, and the average cross-sectional area of these neurones was considerably larger than the mean size of cells sampled from the total population in the same region of the nucleus (Fig. 1*b*), indicating that, in every case, a central portion of the width of area 18 had been successfully injected without involvement of area 17 or 19 (ref. 14).

Tracers were transported to the cell bodies of association neurones in a topographically corresponding part of area 17, in

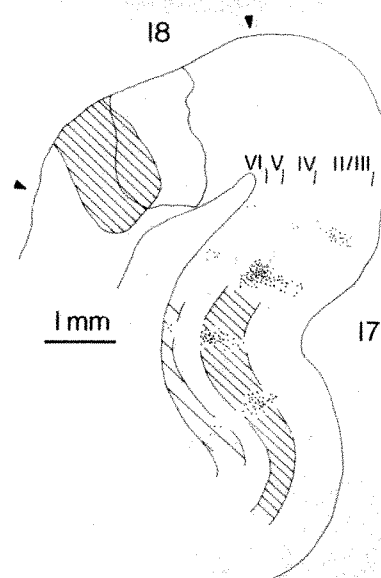


Fig. 3 Camera lucida drawing of a single coronal section through areas 17 and 18 of a 30-day-old kitten that had been injected with FB on day 2 and with DY on day 29. The FB injection site (cross-hatched) overlaps with the DY injection site (unshaded). FB-labelled cells in area 17 are found in two continuous bands (cross-hatched) in the superficial layers and, with lower density, in the deep layers, but DY-labelled cells lie in distinct clusters (dotted), mainly in superficial layers. Where these patches overlap with the band of FB-labelled cells, a number of the DY-labelled cells also contained FB. Areal boundaries were defined on the basis of Nissl- and cytochrome oxidase-stained material; cortical lamination is also indicated.

the same coronal plane as the injection site (Fig. 3); the position of this region of labelling provided yet more evidence that the injections had been placed in the body of area 18. The fact that a region of cortex between the injection and uptake sites contained no labelled cells (Fig. 3) indicated that tracer had not spread directly through the cortex to label neurones in area 17. Invasion of the white matter by diffused tracer around each injection site was slight and, in any case, the pattern of uptake in area 17 was different from that observed when white matter is deliberately injected².

In the kitten that was injected with FB at 2 months old and allowed to survive for 4 weeks, and in the 3-week kitten injected with WGA-HRP and perfused after 24 h (Fig. 2*d*, *b*), tracer was transported into dense clusters of cells in area 17 of ~300 μ m diameter, separated by virtually label-free zones ~300 μ m wide (Table 1 shows average cell densities in the middle of clusters in sections 50- μ m thick). Just as in adult cats, most of the labelled neurones were in the upper cortical layers, with small numbers (one-fifth or less of the density of cells in the upper layers) lying in radially aligned sparse clusters in the lower layers (Table 1). The similarity in the patterns of labelling in area 17 in these two animals suggests that FB was not substantially metabolized and hence lost from cells during the weeks after an injection, nor did it spread widely to label distant cells. Previous workers have demonstrated that FB can remain for long periods within the cytoplasm of a neurone without obvious signs of damage or leakage across the cell membrane: even after several weeks, uptake by neighbouring cells (usually glial cells) is slight^{5,15}. We also saw occasional, very faintly stained cells near neurones that were heavily labelled with FB, but they were small in number, easily distinguished and were not included in our counts of labelled cells.

In younger kittens, aged 1, 4 and 10 days, that had been injected with WGA-HRP and perfused soon afterwards (8 h), the picture was quite different (Fig. 2*a*): there were two continuous bands of labelled cells of uniform density in area 17 in the 1- and 4-day animals, and in most sections from the 10-day

Table 1 Average densities of labelled cells (mm^{-2} , in sections $50\ \mu\text{m}$ thick) in superficial and deep layers of area 17 after injections of tracers into area 18

	Injections followed by short (8 or 24 h) survival					Injections followed by long survival		
	Age at injection (days)					Age at injection		
Cell density in superficial layers	1	4	10	20	29	2 days	2 days	2 months
	170 (21)	462 (43)	239 (14)	363* (27)	390† (23)	331 (18)	375 (25)	382* (23)
Cell density in deep layers	209 (25)	210 (28)	191 (14)	52* (16)	55† (12)	81 (12)	69 (19)	74* (11)
Ratios of densities (superficial/deep)	0.8	2.2	1.3	7.0	7.1	4.1	5.4	5.2

Values are means (with s.e.m. in parentheses). In each of the animals in which labelled cells were distributed in non-patchy bands (that is, kittens injected at 1, 2, 4 and 10 days), 10 consecutive sections ($50\ \mu\text{m}$ thick) through the centre of the area of uptake were selected and the cell density calculated in the upper and lower layers between two lines, 1–1.5 mm apart, drawn parallel to the radial palisades of cortical cells at the point where the density of labelled cells began obviously to decline towards the edge of the area of uptake. In those kittens in which a patchy distribution was seen, densities were measured within a number (* $n=15$; † $n=10$) of corresponding clusters in both superficial and deep layers from several sections covering the centre of the region of uptake. The ratios of the density in superficial layers to the density in deep layers are shown, and lie between 0.8 and 2.2 for all animals aged ≤ 10 days but are much higher in all kittens older than this at the time of perfusion, regardless of the age of injection.

animal. One band lay in superficial layers and the other band, of similar density, in deep layers (densities are shown in Table 1). In the youngest animals, the density of labelled cells was quite constant along each band and labelled cells were not scattered outside the area topographically related in position to the injection site. These facts argue strongly against the possibility that uptake of tracer by axons of passage in the cortex or the white matter had led to the aberrant pattern of labelling in area 17 in young kittens. In some sections from the 10-day-old animal there was evidence of patchiness in the distribution of labelled cells within the superficial and deep layers as the characteristic clustering of the association cells began to appear^{1,2}.

In the two 30-day-old animals that had been injected with FB on postnatal day 2, there was a non-patchy distribution of labelled cells in both superficial and deep layers in area 17 (Fig. 2c), although the density of labelled cells was markedly reduced in the deep layers (Table 1).

Thus, when a long-lasting tracer is injected into area 18 soon after birth, the pattern of label persisting in both superficial and deep layers of area 17 at a much later age remains non-patchy, as in the newborn kitten, despite the fact that the association projection has by this stage actually matured to its typical adult-like patchy form. When DY was injected into area 18 24 h before perfusion in a 29-day-old animal that had previously been injected with FB on day 2, a patchy pattern of DY-labelled cells was seen in addition to the continuous band of FB-labelled neurones in area 17 (Fig. 3). The centre of the DY injection was slightly closer to the 17/18 border than that of the FB injection, and the two stains only partly overlapped each other in area 18. The patches of DY-labelled cells in area 17 were correspondingly displaced towards the 17/18 boundary, compared with the band of FB-labelled neurones. Where the DY patches were superimposed on the continuous FB bands, about one-third of the labelled cells were clearly double-labelled.

As distinct, segregated patches of labelled cells in area 17 were still clearly visible 4 weeks after an injection of FB into a 2-month-old kitten, and since the density of label in 30-day-old animals injected with FB on day 2 was not uniform throughout the depth of the cortex but was virtually absent from the lower half of layer IV (Fig. 2), it is most unlikely that leakage and widespread diffusion of FB had obscured the appearance of patches in the latter animals.

We conclude that many of the cells in the superficial layers of area 17 (as well as some of those in the deep layers) of the neonatal kitten that initially send association projections survive, even though they lose their connections with area 18 by the third week postnatal, to leave the typical patchy distribution of cortico-cortical cells. Our results (Table 1) provide no evidence

for a net postnatal change in the density of association cells within regions of the upper layers of area 17 that remain connected to area 18, and indicate that wholesale death of association cells probably does not occur within the superficial layers. On the other hand, the density of labelled cells in the deep layers of area 17 was much reduced in older kittens and was very similar whether the injection into area 18 had been made on day 2, with long survival, or after 20 days of age. Thus, the loss of the aberrant early projection from the infra-granular layers may partly result from cell death. The marked reduction in cell density in deep layers relative to superficial layers (Table 1) cannot be explained by differential growth of the cortex. First, these observations were all made on the relatively flat portion of area 17, in the medial bank of the lateral gyrus, where areal expansion should stretch all layers roughly equally: in any case, direct measurement of the dimensions of the medial bank of the lateral gyrus suggests that this part of area 17 expands in surface area by only ~15% between days 10 and 20, during which period there is a fourfold or greater fall in the density of labelled cells in the lower layers (Table 1). Measurement of cortical layers II, III and upper IV shows that they actually increase in thickness (by 38% on average) more than do layers V and VI (by 25%) over the first 4 weeks postnatal. Wholesale migration of association neurones from deep to superficial layers is not excluded but is unlikely as very few labelled cells were ever seen in lower layer IV. On balance, our results are most parsimoniously explained by the death of aberrant association neurones in the deep layers.

The cortico-cortical projection from area 17 to area 18 is present and topographically arranged at birth, before the onset of visual stimulation, and is highly exuberant, much of it originating from aberrant populations of cortical neurones. Axonal retraction and cell death both appear to have major roles in the postnatal refinement of this projection, the former leading to the typical clusters of association cells in the upper layers, the latter contributing to the substantial reduction of the projection from the lower layers.

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Ultrastructural localization of choline acetyltransferase in vascular endothelial cells in rat brain

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Furchgott and Zawadzki¹ have shown that acetylcholine (ACh) does not act directly on the smooth muscle of blood vessel walls, but rather via receptors on the endothelial cells lining the lumen, to release an endothelium-derived relaxing factor (EDRF). As it is very unlikely that neurotransmitter released from the periaxillary nerves, which are confined to the adventitial-medial border, diffuses all the way through the medial muscle coat before acting on endothelial cells to release EDRF to produce vasodilatation, this discovery has been regarded as an indication of a pathophysiological mechanism, rather than a physiological one (see refs 2, 3). ACh is rapidly degraded in the blood by acetylcholinesterase, so that ACh must be released locally to be effective on endothelial cells. Here we demonstrate the immunocytochemical localization of choline acetyltransferase in endothelial cells of small brain vessels, which is consistent with the view that the ACh originates from endothelial cells that can synthesize and store it. We suggest that release of ACh following damage to endothelial cells during ischaemia contributes to a pathophysiological mechanism of vasodilatation which protects that segment of vessel from further damage as well as brain cells from hypoxia.

The source of local ACh that might be involved in pathophysiological vasodilatation via endothelial cells is problematical, and it is this aspect that has been examined in the present

investigation of vessels of the brain, where potent ACh-induced vasodilatation has been shown to be dependent on the presence of intact endothelial cells⁴.

Eight adult Wistar rats were anaesthetized with ether and perfused via a cannula tied into the ascending aorta with 200 ml of fixative containing 4% paraformaldehyde, 0.1% glutaraldehyde, 0.1 M lysine-HCl and 0.01 M sodium periodate in 0.1 M phosphate buffer, pH 7.3. This was immediately followed by 500 ml of the same fixative without glutaraldehyde. The brains were removed and placed in fixative for 2 h, then transferred to 0.1 M phosphate buffer and stored overnight at 4 °C. Coronal sections through the visual cortex were cut at 50 µm using a Vibratome and collected in 0.1 M Tris buffer. Sections were processed for immunocytochemical staining of choline acetyltransferase (ChAT) using a recently derived rat monoclonal antibody against ChAT prepared and tested for specificity as described previously^{5,6}. Following treatment with 3,3'-diaminobenzidine and hydrogen peroxide, sections were transferred to 1% OsO₄ for 30 min, stained with 1% aqueous uranyl acetate, dehydrated through an ethanol series and embedded in Araldite. Preincubation of the antiserum with purified ChAT has been shown to abolish cortical staining⁷. In sections from two control rats the primary antibody or the rabbit anti-rat IgG were omitted, resulting in elimination of positive staining in the peroxidase-antiperoxidase procedure.

Examination of the sections under the light microscope and of numerous ultrathin sections from all experimental animals with the electron microscope showed that a small percentage (~10%) of endothelial cells of cortical capillaries and small blood vessels contained ChAT immunoreactivity (Fig. 1). This immunoreactivity was confined to the cytoplasm. Because of the dark nature of the reaction product, organelles present in the cytoplasm could not be discerned, except for mitochondria which appeared to be clustered near the nucleus (Fig. 1c). The relatively small number of labelled endothelial cells may indicate that the levels of ChAT are too low to be detected in some cells and/or that a small proportion of cells containing ACh is sufficient to activate neighbouring cells to release EDRF. ChAT immunoreactivity was also localized in nonpyramidal neurones primarily of the bipolar variety, in agreement with previous findings^{8,9}. Occasionally the dendrites of these neurones, as well as ChAT-stained axons, appeared to be in close association with blood vessels.

The finding that ChAT is localized in vascular endothelial cells in the brain prompts the speculation that choline is taken



Fig. 1 *a*, Longitudinal section through part of a capillary in the upper layers of the rat visual cortex. The wall of the capillary comprises a ChAT-labelled endothelial cell (*) and a process (arrows) of an unlabelled endothelial cell from the same capillary. *b*, Unlabelled endothelial cell from the same capillary. *c*, The labelled cell shows a clear nucleus (Nuc) and thin perinuclear cytoplasm containing a few organelles and a cluster of mitochondria. *a*, *b*, $\times 5,830$; *c*, $\times 15,770$.

up by endothelial cells and converted in the presence of ChAT to be stored as ACh; any local damage to these cells would result in release of ACh and vasodilatation resulting from relaxation of the smooth muscle and/or endothelial cell processes (see ref. 10). Such local vasodilatation mediated by ACh released from damaged endothelial cells would provide protection from further damage in this region of the vascular bed and would protect brain cells from hypoxia.

The number of endothelial cells that contain ChAT in different vascular beds and vessels, and within particular vessels in any one bed, is currently being studied. Preliminary studies have revealed ChAT immunoreactivity in endothelial cells of mesenteric arteries of the rat, but it will be necessary to study ChAT localization in some larger vessels, such as the rabbit thoracic aorta, the tissue that Furchgott used when making the original observations, before it can be claimed that local synthesis, storage and release of ACh from endothelial cells is the universal

mechanism for ACh endothelial cell-mediated vasodilatation. It will also be important to determine how far the ACh released from endothelial cells is conveyed to other parts of the vasculature before being inactivated.

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Glial cells express N-CAM/D2-CAM-like polypeptides *in vitro*

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The joining together of neurites to form fascicles and the growth of axons along glial surfaces^{1,2} during early development suggest that neurone-neurone and neurone-glial adhesion interactions are of considerable importance for defining nerve tracts. *In vitro* studies have indicated that adhesion between neurones³⁻⁵ involves a glycoprotein that has been independently studied under the names of N-CAM (for neural cell adhesion molecule⁶⁻⁸), D2-CAM⁹ and BSP-2 (refs 10, 11). As N-CAM/D2-CAM appears to be a homophilic ligand¹² that binds to N-CAM/D2-CAM polypeptide on adjacent cells, this glycoprotein is potentially important in adhesion interactions between any two N-CAM/D2-CAM-expressing cells. While it has been suggested that neurone-glial adhesion involves molecules other than N-CAM/D2-CAM¹³⁻¹⁵, it is known that N-CAM/D2-CAM antigenic determinants are expressed by glial cells *in vivo*^{16,17} and that injection of anti-N-CAM antibodies into the eye-cup of chick embryos disrupts normal patterns of neuritic apposition to glial endfeet in the developing optic stalk¹⁷. Do the molecules expressed by glia share restricted antigenic determinants, or binding domains, with N-CAM/D2-CAM, or are N-CAM/D2-CAM polypeptides expressed by glia? Here we present immunocytochemical evidence which suggests that all classes of macroglia express N-CAM/D2-CAM antigenic determinants on their surfaces and immunochemical analyses which indicate that the molecules expressed by purified astrocytes are closely similar, or identical, to at least some forms of N-CAM/D2-CAM obtained from whole brain or purified neurones. However, our results also suggest that different N-CAM/D2-CAM polypeptides may be separately expressed by neurones and astrocytes.

Expression of N-CAM/D2-CAM antigenic determinants was examined in cultures of all classes of central nervous system (CNS) macroglia that can be identified with cell-type specific antibodies and on Schwann cells, the glial cells of the peripheral

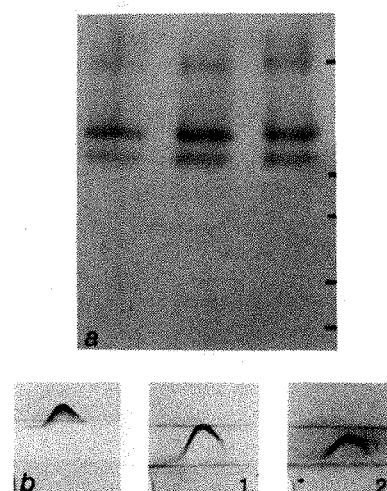


Fig. 1 *a*, Comparison of the anti-D2-CAM (lane 2), anti-N-CAM (lane 3) and anti-BSP-2 (lane 1) antisera in terms of the antigens immunoprecipitated by each from rat brain membranes indicates that all three antisera extract the same three polypeptides from brain. Bars represent the positions of standard markers of M_r : 200,000, 100,000, 69,000, 46,000 and 31,000. *b*, Antibody specificities were compared by crossed immunoelectrophoresis with intermediate gel. The intermediate gel contained no antiserum (left), anti-BSP-2 antiserum (middle) and anti-N-CAM antiserum (right).

Methods. *a*, Anti-D2-CAM antiserum is described in ref. 30 and anti-BSP-2 in ref. 27; anti-N-CAM antiserum was produced by injecting a rabbit with three intraperitoneal injections of 50 μ g of purified mouse N-CAM (purified by affinity chromatography using a monoclonal antibody specific for N-CAM carbohydrate structures²⁵) in Freund's complete adjuvant at 3-week intervals (M.W. and U.R., unpublished). Membranes (from 25-day-old animals) were labelled with ¹²⁵I by the chloramine-T method⁴⁵. Iodinated membranes were solubilized in Tris-barbitol buffer (0.07 M Tris, pH 8.6) containing 4% Triton X-100, 0.2 mM phenylmethylsulphonyl fluoride and 100 U ml⁻¹ aprotinin for 60 min on ice. After centrifugation at 10,000g for 15 min, the supernatants were submitted to crossed electrophoresis⁴⁶ with anti-BSP-2 (lane 1), anti-D2-CAM (lane 2) or anti-N-CAM (lane 3) antibody in the second-dimension gel. Immunoprecipitates were solubilized by boiling for 5 min in buffer containing 2% w/v SDS and 5% v/v 2-mercaptoethanol and analysed by SDS-polyacrylamide gel electrophoresis on 4-20% gradient gels⁴⁷. Gels were dried and fixed for autoradiography on Kodak X-Omat film at -70 °C. *b*, For crossed immunoelectrophoresis⁴⁸, Triton X-100 solubilized rat brain membranes were submitted to crossed immunoelectrophoresis against anti-D2-CAM antiserum. An intermediate gel was included containing the following antisera: (left) no antiserum, (middle) anti-BSP-2 antiserum, and (right) anti-N-CAM antiserum. After electrophoresis the plates were stained for protein with Coomassie stain. Anti-BSP-2 and anti-N-CAM antibodies retracted the D2-CAM immunoprecipitates into the intermediate gel, demonstrating that they recognize the D2-CAM antigen(s).

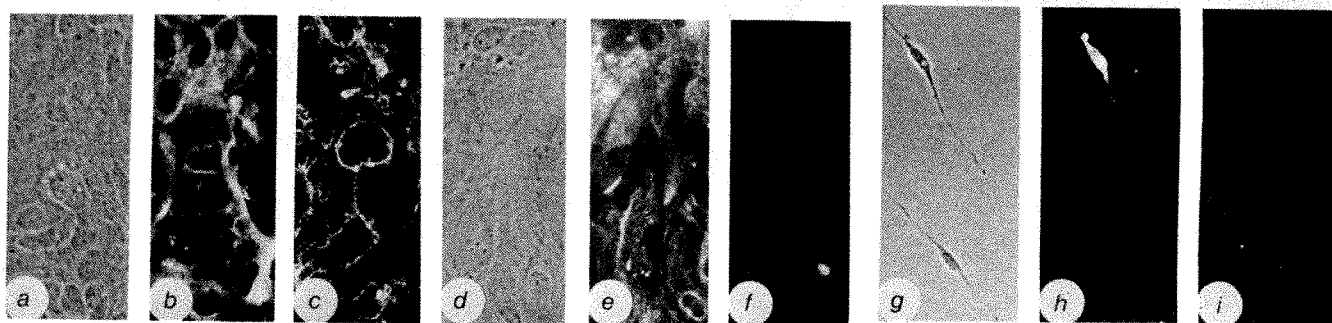


Fig. 2 N-CAM/D2-CAM antigenic determinants are expressed by astrocytes and Schwann cells. *a-c*, Living cultures of purified type 1 astrocytes^{18,19} grown on poly-L-lysine-coated glass coverslips were incubated first with H28 monoclonal antibody (hybridoma supernatant, undiluted), and then with biotin-conjugated goat anti-rat immunoglobulin (Amersham, 1:100), and fluorescein-conjugated streptavidin (Amersham, 1:100). Cultures were then fixed with methanol (-20°C , 10 min) and stained with rabbit anti-glial fibrillary acidic protein (GFAP) antiserum⁴⁹ (1:2,000), followed by rhodamine-conjugated goat anti-rabbit immunoglobulin (R1g-Rd). Identical staining patterns for N-CAM/D2-CAM were obtained by staining the mouse cultures, or similar rat astrocyte cultures, with rabbit anti-N-CAM, anti-D2-CAM or anti-BSP-2 antisera, followed by labelling with goat anti-R1g-Rd. *a*, Phase contrast; *b*, anti-GFAP; *c*, H28 anti-N-CAM. *d-f*, Control staining for *a-c*. H28 antibody was not included in the antibody incubations. *g-i*, Schwann cells from the sciatic nerves of neonatal mice were examined with H28 antibody and with rabbit anti-N-CAM, D2-CAM or BSP-2 antisera. Cultures were labelled as for *a-f*, except that rabbit anti-P₀ antiserum was used in place of rabbit anti-GFAP. N-CAM/D2-CAM antigenic determinants were expressed by many Schwann cells; however, most Schwann cells which had expressed the myelin protein P₀ did not co-express N-CAM/D2-CAM. *g*, Phase contrast; *h*, anti-P₀; *i*, H28. Labelling conditions and analysis of cultures as in refs 18–20. Serum from nonimmune rabbits did not label the surface of astrocytes or Schwann cells, and none of the conjugates used reacted with antibodies from inappropriate species or labelled cells in the absence of anti-N-CAM/D2-CAM or cell-type-specific antibodies.

nervous system. Purified astrocytes from rat and mouse cerebral cortex were prepared according to published procedures^{18,19}. These cultures contained 95–98% type 1 astrocytes²⁴—that is, 95–98% of the cells were labelled with antiserum against glial fibrillary acidic protein (GFAP), a cell-type specific marker of astrocytes²¹, but did not label with the monoclonal antibody A2B5 (ref. 22). These cultures contained no other classes of macroglia, and no neurones (see refs 18, 19). The small proportion of contaminating cells were fibroblast-like cells that expressed cell-surface fibronectin, but did not express GFAP. All immunolabelling and immunochemical experiments were carried out on astrocyte cultures of this kind. In addition, experiments involving biosynthetic labelling or immunochemical quantification were repeated on astrocyte cultures that were prepared by other methods²³ and grown for 3–4 weeks in Dulbecco's modified Eagle's medium containing 10% v/v heat-inactivated horse serum. The two types of astrocyte cultures produced identical results in these experiments. Expression of N-CAM/D2-CAM determinants by other CNS glia was examined in cultures of optic nerves from embryonic chicks and neonatal rats and mice, and expression of these determinants by Schwann cells was examined in enriched Schwann cell cultures prepared from sciatic nerves of neonatal mice (as in ref. 24).

Cultures were examined with three separate rabbit antisera (anti-N-CAM, anti-D2-CAM and anti-BSP-2, see Fig. 1) against the N-CAM/D2-CAM polypeptides, with a monoclonal antibody directed against chick N-CAM²⁵ and the H28 monoclonal antibody directed against mouse N-CAM^{11,26}. N-CAM/D2-CAM is expressed in multiple forms that differ in their carbohydrate composition^{6–8,10} and the size of their cytoplasmic domains²⁷, but that are thought to be otherwise identical^{27,28}. In post-embryonic brain the three dominant forms of this molecule have relative molecular masses (M_r) of 120,000, 140,000 and 180,000 (or 200,000, depending on the gel system used)^{26,29,30}. The three rabbit antisera used in our studies immunoprecipitate all three of these polypeptides from rat brain extract (Fig. 1*a*), both anti-N-CAM and anti-BSP-2 antisera react with the D2-CAM antigens (Fig. 1*b*), and all reactivity of N-CAM/D2-CAM antigen(s) with anti-D2-CAM antisera could be removed from extracts of rat brain by preabsorption with anti-N-CAM or anti-BSP-2 antisera (not shown). Thus, it appears that these three antisera are all directed against identical N-CAM/D2-CAM polypeptides. Both monoclonal antibodies have been previously characterized as recognizing protein determinants specific for the N-CAM/D2-CAM polypeptides^{25,31}.

Using immunofluorescence microscopy, all classes of glia examined were found to express N-CAM/D2-CAM determinants. In cultures of purified type 1 astrocytes from rat cerebral cortex, all of the GFAP⁺ astrocytes were labelled by all three rabbit antisera; purified type 1 astrocytes from mice were similarly labelled, and were also labelled by the H28 monoclonal anti-N-CAM antibody (Fig. 2). In cultures of optic nerve from neonatal rats, examined 1 and 3 days after preparation of the cultures, the rabbit antisera labelled cells possessing the antigenic and morphological phenotypes of oligodendrocytes, type 2 astrocytes, oligodendrocyte-type 2 astrocyte (O-2A) progenitors^{20,32} and type 1 astrocytes (not shown). Cells in cultures of mouse optic nerve were similarly labelled and were labelled by the H28 monoclonal anti-N-CAM antibody (not shown). In cultures of chick optic nerve, the majority of cells were labelled with monoclonal anti-chick-N-CAM antibody (not shown). As optic nerve cultures contain no neurones³², these results suggest that N-CAM/D2-CAM determinants are expressed by all of the major classes of CNS macroglia which can be identified by cell-type specific antibodies, and that these determinants are either expressed *in vivo* or are expressed within 24 h of growth *in vitro*. In addition, in cultures of enriched mouse Schwann cells from sciatic nerves of 3–7-day-old mice (prepared as in ref. 24), we found labelling of some, but not all, cells with the morphology typical of Schwann cells (Fig. 2). In double-labelling experiments using rabbit antiserum against the myelin protein P₀, which labels Schwann cells that have been induced to synthesize myelin-specific proteins³³, many of the P₀⁺ Schwann cells were not labelled by any of the rabbit anti-N-CAM/D2-CAM antisera, or by the H28 monoclonal antibody. Some double-positive cells were present in the cultures, but the majority of N-CAM/D2-CAM⁺ cells with Schwann-cell like morphologies were P₀[−]. Loss of expression of N-CAM/D2-CAM antigenic determinants by myelinating Schwann cells has been seen *in vivo*³⁴, and a comparable loss of N-CAM/D2-CAM expression by older oligodendrocytes has also occasionally been seen *in vitro* (M.N. and I. Sommer, independent unpublished observations). In cultures of sciatic nerve that had not been treated to enrich for Schwann cells, a small proportion (<20%) of cells with fibroblastic morphology were lightly labelled by the H28 monoclonal anti-N-CAM/D2-CAM antibody, but most fibroblast-like cells in these cultures were N-CAM/D2-CAM[−].

Immunofluorescence analyses further support the view that the determinants recognized by indirect immunofluorescence, at least on Type 1 astrocytes, represent N-CAM/D2-CAM. (1) Immunoblots of astrocyte extracts with both anti-N-CAM and

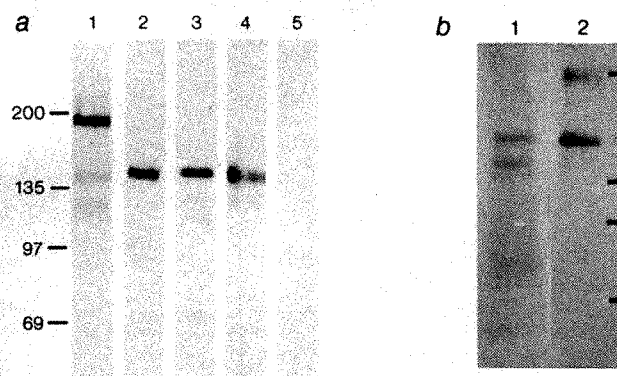


Fig. 3 *a*, SDS-gel electrophoresis of 7-day neonatal mouse brain (1), rat retina (2) or cultured astrocytes (3–5) followed by immunoblotting with anti-N-CAM (1–3), anti-D2-CAM (4) or nonimmune (5) antisera. *b*, Immunoprecipitation of N-CAM/D2-CAM polypeptides from astrocytes and neurones radiolabelled *in vitro* with ^{35}S -methionine. Lane 1, astrocyte cultures; lane 2, cerebellar neurone cultures. *a*, Purified astrocytes were scraped off the surface of Falcon flasks (no. 3024) on ice and centrifuged at 500g for 10 min at 4°C. The cell pellet, or brain tissue, was solubilized in an equal volume of extraction buffer containing 2% SDS and 5% 2-mercaptoethanol, subjected to electrophoresis in 7.5% acrylamide gels, and transferred to nitrocellulose sheets³⁰. N-CAM/D2-CAM was visualized by reaction with the appropriate antiserum followed by horseradish peroxidase-conjugated goat anti-rabbit IgG and staining by treatment with 4-chloro-1-naphthol³¹. M_r s in SDS are based on the migration of myosin, phosphorylase *b*, bovine serum albumin and ovalbumin. Staining is seen of material of M_r 140,000, with very light staining of material of M_r 120,000. *b*, Cultures were labelled with 500 μCi of ^{35}S -methionine (NEN) for a 30-min labelling period, followed by a 30-min chase. Cells were collected, solubilized with Triton X-100 and N-CAM/D2-CAM antigens were immuno-isolated by immunoelectrophoresis against anti-D2-CAM antiserum³⁸. Cultures of cerebellar neurones were established from 8-day-old rats as described by Meier and Schousboe⁵², except that the medium contained 10% v/v horse serum instead of fetal calf serum. After 2 weeks the cultures were labelled with ^{35}S -methionine as described for astrocyte cultures, except that both labelling and chase periods were of 60 min duration. Cells were collected and D2-CAM immuno-isolated as above. Immunoprecipitates were further analysed by SDS-polyacrylamide gel electrophoresis as described in Fig. 1a.

anti-D2-CAM antisera recognized a predominant band of M_r 140,000 that migrated in the same position as the 140,000- M_r band from brain. A weak staining was also observed at M_r 120,000, but this did not reproduce well in the photography (Fig. 3a). (2) In a second protocol, astrocytes and purified neurones³⁵ were separately labelled *in vitro* with ^{35}S -methionine, anti-D2-CAM antiserum was used to immunoprecipitate radio-labelled antigens³⁵, and precipitates were analysed on SDS-polyacrylamide gels (Fig. 3b). This procedure yielded two predominant astrocytic polypeptides, one band of M_r ~140,000, which migrated identically with one of the N-CAM/D2-CAM bands isolated from neuronal cultures, and a second band of M_r ~120,000. A faint band of higher relative molecular mass, which in the gel system used in these particular experiments was of M_r ~200,000, was occasionally observed in extracts prepared from purified astrocytes. N-CAM/D2-CAM immuno-isolated from purified neurones in culture (Fig. 3b, see also ref. 35) also consisted of two bands, but these were of M_r 140,000 and 200,000; no band of M_r 120,000 was detected in neuronal extracts. The polypeptide of M_r 200,000 seen in neuronal extracts is the same polypeptide which in other gel systems, such as that used in Fig. 3a, has a M_r of ~180,000. Thus, in most experiments we were only able to identify the three N-CAM/D2-CAM polypeptides typical of whole brain (M_r = 120,000, 140,000 and 180,000–200,000^{26,29,30}) by considering the bands obtained from astrocytes and neurones in combination. The apparent lack of neuronal synthesis of the 200,000- M_r band

of N-CAM/D2-CAM has also been seen in recent *in vivo* studies in which chick retinal ganglion neurones have been found to transport N-CAM/D2-CAM polypeptides of M_r 140,000 and 180,000, but not of M_r 120,000, along the optic nerve (J. A. Gardner, M.W. and U.R., manuscript in preparation). There is evidence to suggest that different bands of N-CAM/D2-CAM might be transcribed from different messenger RNAs³⁶; it will now be of interest to compare the details of N-CAM/D2-CAM structure, synthesis and processing in neurones and astrocytes.

From the present studies we conclude that glial cells express N-CAM/D2-CAM antigenic determinants on their surfaces, and that purified type 1 astrocytes grown *in vitro* synthesize N-CAM/D2-CAM-like polypeptides. The astroglial molecules we have examined appear to be closely related to N-CAM/D2-CAM polypeptides characterized in extracts of whole brain, and are probably not related to two other putative cell adhesion molecules from the central nervous system, Ng-CAM^{13,14} and L1 (ref. 37), which are thought to be expressed by neurones but not by CNS glia. The three N-CAM/D2-CAM polypeptides isolated from whole brain are thought to differ primarily in the length of their cytoplasmic domains²⁷, and have previously been found to be almost identical as judged by peptide mapping^{27,28,38}. In contrast, peptide maps of L1 and Ng-CAM are markedly different from those of N-CAM/D2-CAM^{14,39}. L1 and Ng-CAM are also immunochemically distinct from N-CAM, although there may be some shared expression of at least one epitope that has been identified by monoclonal antibodies^{14,40}. Moreover, although L1 has an M_r of ~140,000 and Ng-CAM an M_r of 135,000, which are similar in size to one of the astroglial N-CAM/D2-CAM bands, L1 and Ng-CAM antibodies do not detect material of M_r 120,000, the size of the second major astroglial N-CAM/D2-CAM band.

Although in our present studies we have consistently seen high levels of N-CAM/D2-CAM expression by a variety of glial cell types, previous investigations have suggested that glia do not express N-CAM/D2-CAM *in vitro*^{13,14,26}. We do not yet know the reasons for these differing results; however, it seems likely that expression of N-CAM/D2-CAM by astrocytes can be modulated during development, as appears to be the case for muscle cells^{41,42} and Schwann cells. Nothing is yet known about the regulatory influences that might induce the disappearance of N-CAM/D2-CAM from astrocytes.

The hypothesis that neurones are more adherent to glia than to other neurones or to non-glia has been suggested as providing a cellular explanation for the observations that, both *in vivo* and *in vitro*, growing neurites exhibit a marked affinity for glial surfaces^{18,43,44}. For example, during embryonic development of the optic stalk, neuritic growth cones are found predominantly apposed to endfeet of primitive glia and neuritic fascicles are found organized on these glial surfaces but not on other available surfaces¹. The demonstration of N-CAM/D2-CAM on glial cell surfaces raises the question of whether this molecule is involved in such adhesive interactions between neurones and glia, in distinction from the hypothesis that neurite-neurite adhesion is mediated by N-CAM/D2-CAM and neurone-glial adhesion is separately mediated by Ng-CAM^{14,15}. It has recently been found that N-CAM/D2-CAM antigenic determinants are expressed on glial endfeet in the chick optic stalk and that injection of anti-N-CAM antibody into the eye-cup of chick embryos results, within the optic stalk, in a separation of neuritic fascicles from these glial surfaces¹⁷. Thus, in this particular case, the affinity of neurites for glial surfaces may be due to glial expression of N-CAM/D2-CAM. However, the specific contributions of N-CAM/D2-CAM, Ng-CAM and perhaps other molecules to the generally observed affinity between neurones and glia have yet to be determined.

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Note added in proof: Independent experiments by G. Keilhauer, A. Faissner and M. Schachner (*Nature* 316, 728-730: 1985) have also indicated that purified astrocytes express N-CAM/D2-CAM-like in polypeptides. These experiments also indicate that the binding of neurones to astrocytes *in vitro* can be inhibited by anti-N-CAM/D2-CAM antiserum.

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Differential inhibition of neurone-neurone, neurone-astrocyte and astrocyte-astrocyte adhesion by L1, L2 and N-CAM antibodies

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The cell adhesion molecules L1, N-CAM and Ng-CAM have been implicated in cell-cell interactions among developing neural cells¹⁻⁴. L1 and N-CAM are structurally and functionally distinct molecular entities and act synergistically in mediating Ca²⁺-independent adhesion between re-aggregating early postnatal cerebellar cells⁵. N-CAM has been reported to be neurone-specific in the chicken and to mediate fasciculation of neurites and of nerve-muscle interactions. L1, which in the central nervous system has been found only on post-mitotic neurones, mediates migration of granule cell neurones in the mouse cerebellar cortex⁶. In view of the molecules' distinct effects on cell interactions, we wondered whether different neural cell types are involved in the actions of each molecule. Here we report that L1 antigen promotes neurone-neurone adhesion. N-CAM, which is expressed on both neurones and glia, mediates neurone-neurone, neurone-astrocyte and astrocyte-astrocyte adhesion. The L2 carbohydrate epitope shared between the two adhesion molecules⁷ seems to be involved in neurone-astrocyte and astrocyte-astrocyte adhesion and acts in a more than additive manner in N-CAM-mediated neurone-neurone adhesion.

Homogeneous populations of neurones and astrocytes from early postnatal mouse cerebellum were isolated, probed for expression of adhesion molecules (Figs 1, 2) and used to measure adhesion of single-cell suspensions of probe to monolayer target cells in the absence and presence of Fab fragments (Table 1). In all combinations of probe to target cells (neurone-neurone, neurone-astrocyte, astrocyte-neurone and astrocyte-astrocyte),

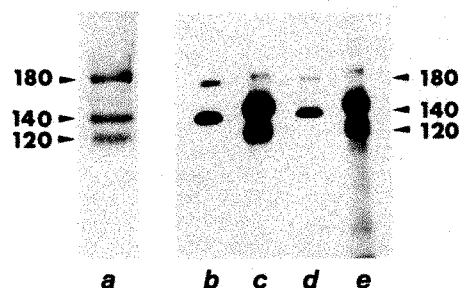


Fig. 1 Double immunofluorescence labelling of monolayer cultures from 5-day-old C57BL/6J mouse cerebellum after 2 days *in vitro*. Polyclonal N-CAM antibody (a), prepared as described previously³, reacts with vimentin-positive (b), fibronectin-negative glial cells. Note the patchy distribution of N-CAM on the cell surface (a). c, Phase-contrast micrograph of fluorescence images a and b. Polyclonal N-CAM antibody (d) reacts with an O4 antigen-positive oligodendrocyte (e). f, Phase-contrast micrograph of fluorescence images d and e. Cell culture, cell type-specific markers and double immunofluorescence procedures have been described previously¹⁹. Immunofluorescence labelling of GFAP-positive astrocytes was weaker than that of less differentiated, GFAP-negative, vimentin-positive astrocytes. The tendency for N-CAM to be more strongly expressed on less differentiated glia was also seen with oligodendrocytes: more than 80% of all O4 antigen-positive cells, but only less than 5% of all O1 antigen-positive cells were N-CAM-positive. $\times 300$.

antibodies to liver membranes, which react well with cell surfaces of astrocytes and neurones⁶, did not inhibit adhesion, whereas N-CAM antibodies did. Polyclonal L1 antibodies interfered with neurone-neurone adhesion, but not with neurone-astrocyte or astrocyte-astrocyte adhesion, even when astrocytes from different postnatal ages, from different brain regions or maintained *in vitro* for varying time periods were used. Monoclonal L2 antibodies did not reduce neurone-neurone adhesion, but decreased neurone-astrocyte and astrocyte-astrocyte adhesion. Astrocyte-neurone adhesion resulted in slightly different inhibition values from those seen in the reciprocal combination, as has been observed in other adhesion systems⁸.

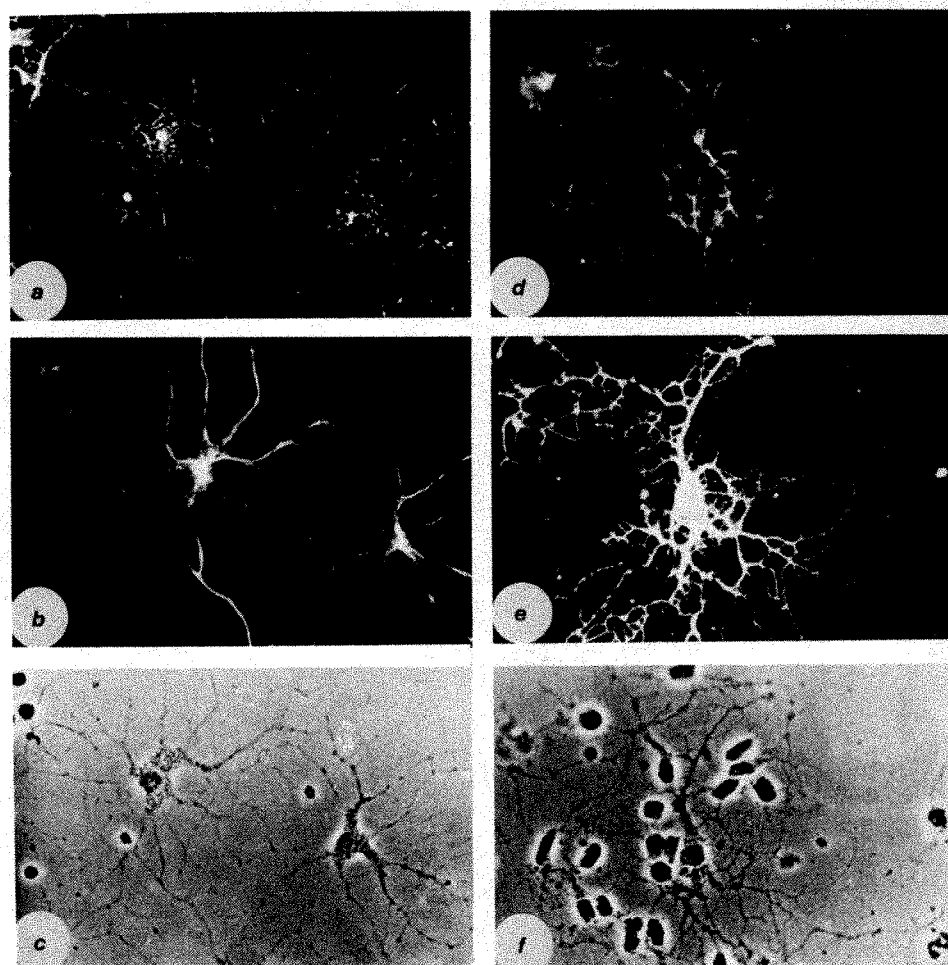
Inhibition values obtained with mixtures of polyclonal L1

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Fig. 2 Immunoprecipitation of N-CAM from cultures of isolated neurones and astrocytes from early postnatal mouse cerebellum using poly- and monoclonal N-CAM antibodies. **a**, Western blot with polyclonal N-CAM antibody ($10 \mu\text{g ml}^{-1}$ IgG fraction) on crude membrane fraction from adult mouse brain resolved by SDS-polyacrylamide gel electrophoresis (PAGE) on a 6–12% gradient gel⁵. Three bands are seen in the relative molecular mass regions of 180,000 (180K), 140K and 120K. **b–e**, Fluorograph of immunoprecipitates from ³⁵S-methionine-labelled cultures of neurones (**b, d**) and astrocytes (**c, e**) with polyclonal N-CAM (**b, c**) and monoclonal N-CAM antibody H28.123 (**d, e**). Polyclonal and monoclonal antibodies gave identical results. In neurone cultures the antibodies detected two bands of 180K and 140K. In astrocyte cultures two prominent bands in the 140K and 120K range were seen and a fainter band in the 180K range. Note the apparently higher molecular weight of the 180K and 140K forms in astrocytes compared with neurones.

Methods. Cells were obtained and maintained as described in the legend to Table 1. Cultures of 1.5×10^7 cells per 6-cm Petri dish were incubated with $200 \mu\text{Ci } ^{35}\text{S}$ -methionine (Amersham; $1,000 \text{ mCi mmol}^{-1}$) per ml in methionine-free minimal essential medium containing 10 mM HEPES and 2 mM glutamine for 4 h at 37°C.

After two washes with HBSS, cells were solubilized by adding 1 ml of solubilization buffer (150 mM NaCl, 20 mM Tris-HCl, 1 mM EDTA, 1 mM EGTA, 10 mM methionine, 0.5% Nonidet P-40, 0.05% NaN_3 , pH 7.4), the protease inhibitors aprotinin, soybean trypsin inhibitor and turkey egg white trypsin inhibitor (all at $10 \mu\text{g ml}^{-1}$), phenylmethylsulphonyl fluoride and iodoacetamide (both at 5 mM), pepstatin and leupeptin (both at $2 \mu\text{M}$). After 30 min on ice, cells were scraped from the dish, nuclei and debris removed by centrifugation for 10 min at 800g and supernatants cleared for 45 min at 100,000g. For immunoprecipitation, aliquots (1 ml) of the supernatants were incubated with polyclonal N-CAM (150 μg IgG fraction) or monoclonal N-CAM antibody H28.123 (ref. 23; $10 \mu\text{g}$ IgG fraction) for at least 1 h at 4°C. Then 150 μl protein A-Sepharose (10% v/v; Sigma) was added to rabbit IgG and 70 μl of a 10% v/v suspension of Sepharose 4B beads coupled with mouse monoclonal antibody to rat κ -chain²⁴ to monoclonal H28.123 antibody, all in solubilization buffer. After 1 h at 4°C, the beads were centrifuged through a sucrose cushion, washed several times with solubilization buffer and boiled for 4 min at 100°C in 45 μl of SDS-PAGE sample buffer⁵. All steps were carried out at 4°C. Precipitates were analysed by SDS-PAGE on 6–12% gradient gels⁵. Gels were then fixed in 10% acetate, 30% methanol in water and treated with 'amplify' as recommended by the producer (Amersham). Kodak X AR-5 X-ray films were exposed for various times at -70°C and developed according to the supplier's instructions.



and N-CAM antibodies were not additive, indicating that the two adhesion mechanisms are not independent in this adhesion system. In the presence of L2 antibody, polyclonal N-CAM antibodies were more than additive in inhibiting neurone-neurone, but not neurone-astrocyte or astrocyte-astrocyte adhesion. In contrast, inhibition by L2 antibody was unaffected by monoclonal N-CAM antibody, which by itself did not interfere with adhesion (not shown, see also ref. 3). Also, L2 antibody did not increase polyclonal L1 antibody-induced inhibition of neurone-neurone or neurone-astrocyte adhesion (not shown). In the presence of the three antibodies—polyclonal N-CAM and L1 antibodies and monoclonal L2 antibodies (each at a concentration of 1.0 mg ml^{-1})—the inhibition of neurone-neurone adhesion did not exceed the values obtained with N-CAM and L2 antibodies alone. Adhesion of neurones and astrocytes to skin fibroblasts was not reduced by N-CAM, L1 or L2 antibodies, regardless of whether fibroblasts were used as target or probe cells.

Our study has shown that distinct adhesion mechanisms operate between neurones and astrocytes in the mouse cerebellum at the time of postnatal granule cell migration: (1) L1 antigen is involved in neurone-neurone interaction. (2) N-CAM not

only mediates neurone-neurone, but also neurone-astrocyte and astrocyte-astrocyte adhesion. All cell types involved express N-CAM on their surfaces. (3) The L2 domain is involved in an adhesive function that is manifest by itself in neurone-astrocyte and astrocyte-astrocyte and, in conjunction with N-CAM, in neurone-neurone adhesion.

Neurone-glia interaction has been postulated to underlie the migration of granule cell neurones in the early postnatal cerebellum⁹. Because L1 antibodies interfere with migration⁶ and seem to block only neurone-neurone adhesion, it is possible that a prerequisite for migration is an L1-mediated sorting out of post-mitotic granule cell bodies or extension of granule cell axons. On the other hand, it is conceivable, although unlikely, that the cultured neurones and astrocytes used in the present study are not representative of their *in vivo* counterparts. Although L1 antigen shares certain properties with the neurone-neurone and neurone-glia adhesion molecule Ng-CAM^{4,10}, the present study failed to detect the molecular domains involved in neurone-glia adhesion. It is therefore conceivable that the polyclonal L1 and Ng-CAM antibodies recognize different sets of functional sites on L1 antigen from mouse and Ng-CAM from chicken.

Table 1 Inhibition of adhesion between enriched populations of neurones and astrocytes from early postnatal mouse cerebellum in the presence of Fab fragments from L1, N-CAM and L2 antibodies

Antibody	Ref.	Fab concentration (mg ml ⁻¹)	Neurone* to neurone†	Neurone* to astrocyte†	Astrocyte* to astrocyte†	Astrocyte* to neurone†
None		0	0 ± 3	0 ± 2	0 ± 2	0 ± 4
Poly-liver	5	1.0	2 ± 4	-3 ± 2	1 ± 1	-2 ± 3
Poly-L1	8	1.0	27 ± 4	0 ± 3	-1 ± 2	0 ± 2
Poly-N-CAM	3, 4	1.0	40 ± 7	29 ± 7	24 ± 2	28 ± 5
		2.0	41 ± 3	32 ± 3	23 ± 4	26 ± 12
Mono-L2	6	1.0	1 ± 5	25 ± 10	15 ± 3	15 ± 8
Poly-L1 + poly-N-CAM		0.5 + 0.5	36 ± 4	ND	ND	ND
		1.0 ± 1.0	36 ± 2	ND	ND	ND
Poly-N-CAM + mono-L2		1.0 ± 1.0	56 ± 3	34 ± 6	26 ± 3	ND

Single-cell suspensions from early postnatal C57BL/6J mouse cerebella¹⁹ were used for isolation of astrocyte- and neurone-enriched cell populations. Small cerebellar neurones were obtained from 6-8-day-old mice by centrifugation (10 min, 4 °C, 1,200g) through Percoll (Pharmacia, 40.5% in Ca²⁺- and Mg²⁺-free Hank's balanced salt solution (CMF-HBSS) containing 10 mM HEPES and 0.1% bovine serum albumin pH 7.2). The pellet of cells was washed once in basal Eagle's medium with Earle's salts containing 10% horse serum (culture medium) and seeded in poly-L-lysine-coated Costar plates at a density of 1×10^6 in 0.5 ml culture medium per well. Monolayers were used as target cells after 3 days in culture with >99% of all cells expressing N-CAM or L1 antigen and ~60% the L2 epitope. The percentage of glial fibrillary acidic protein (GFAP)- and vimentin-positive astrocytes after 3 days in culture was <1%, compared with 6 ± 2% GFAP-positive and 8 ± 2% GFAP-positive and 8 ± 2% vimentin-positive astrocytes in cultures made from whole cerebellum. Astrocytes were obtained from 3-5-day-old mice by seeding single-cell suspensions into T75 tissue culture flasks (Nunc) at a density of 30×10^6 cells in 10 ml culture medium per flask. After 3 days, cultures were treated with cell-surface-reactive monoclonal antibodies M5 directed against all cerebellar neurones (C. Lagenaur and M.S., in preparation), 04 directed against more and less differentiated oligodendrocytes²⁰, and MESA-1 directed against fibroblast-like cells of endothelial origin²¹ in the presence of guinea pig complement (diluted 1:10). Residual cells were treated with 100 µg ml⁻¹ trypsin, 1 mM EDTA in CMF-HBSS for 5 min at room temperature, centrifuged (5 min, 4 °C, 60g) and seeded in poly-L-lysine-coated Costar plates (10^5 cells in 0.5 ml culture medium per well). Monolayer cultures were used as target cells after 1 day in culture with >97% of all cells expressing GFAP and N-CAM and ~60% the L2 epitope. Fibroblasts were obtained from the skin of 1-day-old mice by treatment with collagenase IV and trypsin²². No significant cell death or proliferation was observed within the culture periods studied. Probe cells were obtained from monolayer cultures of enriched neurones (after 1 day in primary culture), astrocytes (1 day after immunocytolysis and subsequent subculture) and fibroblasts (3 days in primary culture) by treatment with 20 µg ml⁻¹ trypsin, 1 mM EDTA in CMF-HBSS for 15 min at room temperature after three washes with CMF-HBSS containing 1 mM EDTA. To vitally stain the probe cells, fluorescein diacetate (10^{-6} M) was included during the trypsinization step. Trypsinization was stopped by adding 40 µg ml⁻¹ soybean inhibitor and cells were collected by centrifugation (10 min, 40 °C, 60g). Treatment with the low trypsin concentration did not visibly reduce the intensities of immunofluorescence staining with N-CAM, L1, L2 and liver membrane antibodies compared with non-trypsinized cells. Cell concentrations were adjusted to 4×10^5 cells ml⁻¹ CMF-HBSS for neurones, and to 1×10^5 cells ml⁻¹ for astrocytes. Probe and target cells were treated with 0.5 ml Fab fragments of antibodies in CMF-HBSS for 20 min on ice before the adhesion test. Target cells were washed three times with CMF-HBSS containing 1 mM EDTA before addition of Fab fragments. Probe cells (2×10^5 neurones or 0.5×10^5 astrocytes in 0.5 ml) were added to target cells in Costar wells and incubated for 30 min at room temperature in a reciprocal shaker (Infors, Basel, Switzerland) at 40 cycles per min. Unbound probe cells were removed from the monolayer target cells by three gentle washings in CMF-HBSS (1 ml for each well and washing). Aggregation among probe cells was negligible both in controls and in the presence of Fab fragments. Adhering cells were scored by counting ~1,000 cells in >10 microscopic fields at $\times 100$ magnification with an inverted fluorescence microscope (ICM 405, Zeiss). Without Fab fragments the binding of the total input cells was $79 \pm 3\%$ (897 ± 34.0 cells mm⁻²) for neurone-neurone, $82 \pm 2\%$ (931 ± 22.7 cells mm⁻²) for neurone-astrocyte, $47 \pm 4\%$ (133 ± 11.3 cells mm⁻²) for astrocyte-astrocyte, and $49 \pm 2\%$ (138 ± 5.6 cells mm⁻²) for astrocyte-neurone adhesion. Using fibroblasts, $10 \pm 5\%$ binding was seen in all combinations with neurones and astrocytes. Binding of probe cells was predominantly to target cells and not to the cell-free substrate. Each experimental value was run in quadruplicate. Per cent inhibition of adhesion in the presence of Fab fragments was calculated by: % inhibition = [adhesion (control) - adhesion (+Fab)/adhesion (control)] $\times 100$. Numbers are mean values for several experiments $\pm$ s.d. Eight experiments were performed for neurone-neurone and neurone-astrocyte, and four experiments for astrocyte-neurone and astrocyte-astrocyte adhesion. The difference in inhibition of adhesion given by antibody combinations and individual antibodies is significant for the combination polyclonal N-CAM and monoclonal L2 only for neurone-neurone adhesion ($P < 0.001$, Student's *t*-test). Specifications of antibodies and preparations of Fab fragments have been described previously and appropriate references are given. ND, not done.

* Probe cells; † target cells.

N-CAM has previously been detected on glia in addition to neurones (refs 3, 11, 12; see also accompanying paper¹³), although its function in neurone-glia adhesion has remained elusive¹⁰. As N-CAM has been implicated in a homophilic 'self-aggregating' adhesion mechanism¹⁴, it is expected that N-CAM-positive glial cells adhere to other N-CAM-positive cells. In the conditions of our study, we did not observe the synergistic effect between L1 and N-CAM molecules which was seen in re-aggregating cultures of single cells⁵. It is likely that the non-additive effects are the result of different cell dissociating procedures, the lower temperature used to prevent self-aggregation among probe cells, and the anchoring of the target cells at the substrate surface, thereby reducing the molecules' lateral mobility within the membrane and cooperative effects between them.

Adhesion affinities of N-CAM are differentially modulated by the carbohydrate chains of the adult and embryonic forms^{14,15}. It has been suggested that carbohydrate chains are involved in stabilizing the conformation of glycoproteins, and thus indirectly affect their function^{16,17}, or that they are more directly involved as ligands for lectin-like receptors¹⁸. The L2 domain may be yet another, functionally important carbohydrate moiety. The function of the L2 epitope on some but not all N-CAM molecules of the adult and embryonic forms remains to be elucidated in the intact developing nervous system.

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Functional reconstitution of purified muscarinic receptors and inhibitory guanine nucleotide regulatory protein

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Muscarinic receptors trigger several different responses including an increase in concentration of cyclic GMP, a decrease in cyclic AMP concentration, breakdown of polyphosphoinositides and changes in ion permeability. It is not yet clear whether these reactions occur sequentially or independently and which directly coupled to the muscarinic receptor. Several lines of evidence indicate that muscarinic receptors in many, if not all, cell types are coupled to the inhibitory guanine nucleotide regulatory protein (N_i or G_i) of adenylate cyclase. To provide direct evidence for this coupling, we have reconstituted muscarinic receptors purified from porcine brain with N_i purified from rat brain in a phospholipid vesicle. Here, we report that the GTPase activity of N_i is stimulated by carbachol. This action is blocked by the simultaneous addition of atropine and is not observed when the N_i protein is ADP-ribosylated. We conclude that one function of the muscarinic receptor is the activation of N_i .

Either the receptor-linked inhibition of membrane adenylate cyclase or the decrease in cellular cAMP is reversed¹⁻³ by islet-activating protein (IAP, pertussis toxin⁴⁻⁶), which abolishes the function of N_i ^{2,7-9} as a result of ADP-ribosylation of the protein^{10,11}. Guanine nucleotides also markedly reduce the apparent affinity of membrane receptors for agonists but not for antagonists¹²⁻¹⁵, and the effect of guanine nucleotides is abolished by IAP^{2,3}. Finally, the guanine nucleotide-sensitive agonist binding component is larger than the nucleotide-insensitive antagonist binding component^{16,17}.

In the present study we purified muscarinic receptors from the digitonin extract of porcine brain by chromatography with an affinity gel of a specific ligand, 3-(2'-aminobenzhydryloxy)tropane¹⁸. The purified protein bound 3 nmol mg^{-1} of ³H-N-methylscopolamine (³H-NMS), representing a 3,000-fold purification. The protein was iodinated and submitted to SDS-polyacrylamide gel electrophoresis (SDS-PAGE). We found a major band with an apparent relative molecular mass (M_r) of 70,000, together with several minor bands (Fig. 1). The major band was the only band detected on rechromatography¹⁹, and proved to be the muscarinic receptor itself or its ligand-binding subunit, as it was also labelled with ³H-propylbenzylcholine mustard, an irreversible muscarinic ligand. The receptor protein purified in this way did not display GTP binding or hydrolysing activity, nor did it serve as a substrate of IAP-catalysed ADP-ribosylation.

GTP-binding proteins serving as substrates of IAP-catalysed ADP-ribosylation were purified from cholate extract of rat brain by sequential application of column chromatographic techniques using DEAE-Sephacel, Ultrogel AcA-34 and hydroxylapatite^{20,21}. The proteins freed of stimulatory guanine nucleotide regulatory protein (N_s) after passage through the final column were a mixture of two IAP substrates, as reported for bovine brain proteins^{22,23}; these were further separated on a column of DEAE-Toyopearl 650(S)²⁰. One of these heterotrimers consists of an α -, a β - and a γ - (M_r 41,000, 35,000 and ~10,000, respectively) and was used as purified N_i for the reconstitution experiments described below. No ligand for muscarinic receptors bound specifically to this protein.

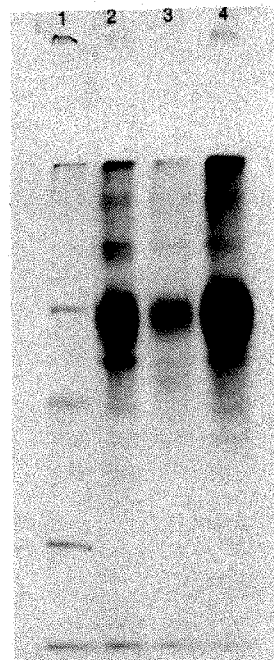


Fig. 1 SDS-PAGE of iodinated receptor preparations used for reconstitution studies. Receptor preparations obtained in three independent preparations are in lanes 2-4. Lane 1, relative molecular mass marker proteins (bovine serum albumin, 67,000; ovalbumin, 45,000; and carbonic anhydrase, 30,000).

Methods. About 900 ml of a digitonin(1%)/cholate(0.1%) extract of membrane preparations from porcine cerebrum were passed through the ABT-agarose column (3 × 43 cm, 300 ml). The ABT-agarose column was washed with 1.5 l 0.2 M NaCl/20 mM K-phosphate buffer (pH 7.0)/0.1% digitonin, then a small column of hydroxylapatite (1 ml, 0.8 × 2 cm) was connected to one end of the ABT-agarose column. Muscarinic receptors were eluted from the ABT-agarose column with buffer containing 0.1 mM atropine and allowed to bind to the hydroxylapatite. After washing the hydroxylapatite column with 0.15 M K-phosphate buffer/0.1% digitonin, muscarinic receptors were eluted with 0.5 M K-phosphate buffer/0.1% digitonin. The preparation contained 265 pmol of muscarinic receptors, with a specific activity of 3,060 pmol mg^{-1} of protein in a volume of 6 ml. The amounts of muscarinic receptors and proteins were assessed by binding of ³H-NMS and with fluorescamine³³, respectively. The purified preparation was radioiodinated by the chloramine T method³⁴ and electrophoresed by the method of Laemmli³⁵, followed by autoradiography.

The muscarinic receptor and N_i proteins thus purified were reconstituted into phospholipid vesicles essentially by the method used for β -adrenergic receptors and N_s ²⁴. A mixture of these proteins with the crude brain phospholipid fraction and phosphatidylcholine in detergents, was submitted to gel filtration to obtain detergent-free phospholipid vesicles in the void-volume fraction. About 80% of the ³H-NMS-binding activity of the receptor protein and 50% of the GTPase activity of N_i was recovered in the vesicles. The fraction of the protein-bound ³H-NMS trapped by a membrane filter (Toyo Roshi TM2) increased from 12 to 43% on reconstitution, reflecting incorporation of the protein into the reconstituted vesicles.

N_i displayed Mg^{2+} -dependent GTPase activity both in phospholipid vesicles (Fig. 2) and in 0.1% Lubrol solution²⁰. Carbachol had no effect unless the receptor protein was also reconstituted into the N_i -containing vesicles; it increased the GTPase activity of N_i at all the concentrations of Mg^{2+} studied (0.15–15 mM). The degree of increase caused by the agonist was larger at lower concentrations of Mg^{2+} ; the affinity of N_i for Mg^{2+} , an activator of the GTPase, was enhanced by stimulation of muscarinic receptors in the reconstituted vesicles (Fig. 2).

Carbachol increased GTPase activity in the receptor- N_i vesicles in a concentration-dependent manner (Fig. 3): the half-maximal effective concentration (EC_{50}) was 3.6 μ M in the presence of 2.8 mM $MgCl_2$. This value was virtually the same as the

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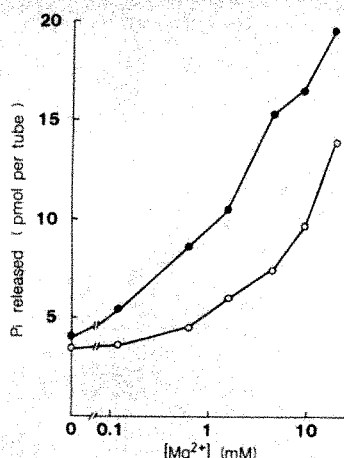


Fig. 2 Magnesium-dependent GTPase activity in receptor- N_i vesicles with or without carbachol. Concentration of free Mg^{2+} is shown on the abscissa. The incubation medium was either further supplemented with 0.1 mM carbachol (●) or contained no carbachol (○).

Methods. N_i (90 μ g or 1 nmol) in 0.7 ml (pH 8.0) of buffer containing 35 mM HEPES, 0.55 mM EDTA, 1 mM dithiothreitol (DTT), 50 mM NaCl, 125 mM K-phosphate buffer, 0.75 mM $MgCl_2$ and 0.05% sodium cholate, was mixed with muscarinic receptor (1.2 μ g or 16 pmol) in 0.7 ml 0.5 M K-phosphate buffer, 0.07% digitonin, 0.7 ml of 0.5 mg ml^{-1} dimyristoyl phosphatidylcholine, 1 mg ml^{-1} brain extract (Folchi Fraction I, Sigma), 3.6 mg ml^{-1} sodium deoxycholate and 0.4 mg ml^{-1} sodium cholate, and reconstituted by gel filtration through a Sephadex column (1.2 $\times$ 25 cm) according to Brandt *et al.*²⁶. The column was prewashed and eluted with 20 mM HEPES, 1 mM EDTA, 1 mM DTT, 0.1 M NaCl and 1.5 mM $MgCl_2$. Vesicles (20 μ l per tube) prepared by this method were incubated at 30 °C for 30 min with 0.5 μ M [γ -³²P]GTP, 15 mM HEPES, 0.75 mM EDTA, 0.75 mM DTT and 75 mM NaCl/ $MgCl_2$ in a total volume of 0.1 ml per tube.

EC_{50} (3.8 μ M) for the carbachol-induced increase in GTPase activity of rat striatal membranes²⁵. The effect of carbachol was antagonized in a concentration-dependent manner by the simultaneous addition of atropine (Fig. 3). The dissociation constant for the atropine/receptor complex was calculated to be 7 nM from the data in Fig. 3 by assuming that atropine competed with carbachol for the same sites on the receptor molecules responsible for increasing the GTPase activity of coupled N_i . This value was virtually the same as the apparent dissociation constant (7.8 nM) for atropine-induced displacement of ³H-NMS from the receptor proteins in a detergent solution¹⁹.

We also determined the potency of carbachol in displacing ³H-NMS bound to the reconstituted vesicles. The apparent dissociation constant (180 μ M) obtained was much larger than the EC_{50} (3.6 μ M) for the GTPase activity. Presumably, agonists bind with a high affinity to a small fraction of receptor proteins that are coupled to N_i , and bind with a low affinity to the residual bulk of uncoupled receptors. It is reasonable to assume that the affinity of muscarinic receptors for agonists is enhanced by their coupling to GTP-binding proteins such as N_i ¹²⁻¹⁵.

N_i purified from rat brain was ADP-ribosylated when incubated with the A-protomer^{5,6} of IAP, the active component of this A-B toxin⁴, in the presence of NAD²⁰. The ADP-ribosylated N_i exhibited the same Mg^{2+} -dependent GTPase activity as did the native (non-ADP-ribosylated) N_i in Lubrol solution or reconstituted phospholipid vesicles. There was no increase in GTPase activity, however, when carbachol was added to the vesicles into which the ADP-ribosylated N_i , instead of the native N_i , was reconstituted together with muscarinic receptors (data not shown). The direct ADP-ribosylation of N_i in the reconstituted vesicles was then achieved in the same experimental conditions as in Figs 2 and 3 by further adding 0.6 mM NAD alone (control) or NAD plus 0.2 μ g of the A-protomer of IAP (ADP-ribosylated) to the GTPase assay mixture. The assay time was extended to 60 min to allow ADP-ribosylation to proceed in the vesicles. The addition of 0.1, 1, 10, 100 and

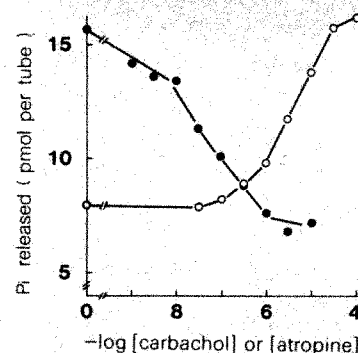


Fig. 3 Concentration-dependent increase of GTPase activity by carbachol in the absence of atropine (○) and antagonism by atropine with 33 μ M carbachol (●). Vesicles containing N_i and muscarinic receptor were prepared and incubated in a medium supplemented with 2.8 mM $MgCl_2$ as described in Fig. 2 legend.

1,000 μ M carbachol produced increases of 0, 10, 48, 86 and 112%, respectively in GTPase activity of the control vesicles (supplemented with NAD alone) and increases of 0, 0, 12, 27 and 41%, respectively, in ADP-ribosylated vesicles (NAD plus the A-protomer of IAP). The A-protomer had no effect in the absence of NAD. Thus, ADP-ribosylation by IAP interfered with the ability of N_i to couple to muscarinic receptors in the reconstituted vesicles.

In conclusion, muscarinic receptors purified from porcine brain are coupled to N_i , the IAP substrate, in phospholipid vesicles in such a manner that stimulation of the receptors by agonists gives rise to increases of the GTPase activity, or of the affinity for Mg^{2+} , of this GTP-binding protein. Subdivision of muscarinic receptors has been proposed based on tissue and agonist specificities²⁶. Two subtypes of muscarinic receptors in embryonic chick heart cells have been reported, one linked to adenylate cyclase inhibition and the other to phosphoinositide breakdown²⁷. In addition, a third type of muscarinic receptors in human astrocytoma has been reported^{28,29} which is coupled to phosphoinositide metabolism, rather than adenylate cyclase, via a GTP-binding protein that did not serve as the substrate of IAP-catalysed ADP-ribosylation, although receptor-linked activation of phospholipid metabolism and Ca^{2+} mobilization was mediated by an IAP substrate in guinea pig neutrophils³⁰ and rat mast cells^{31,32}. It remains to be shown whether the rat brain N_i coupled to muscarinic receptors is responsible for inhibition of adenylate cyclase or activation of phospholipase C under physiological conditions. Direct evidence for the receptor subtypes that interact with different effectors, and molecular characterization of these interactions should be obtained by further studies using reconstitution of purified proteins.

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Primary structure of the α -subunit of *Torpedo californica* ($\text{Na}^+ + \text{K}^+$)ATPase deduced from cDNA sequence

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Sodium- and potassium-dependent ATPase [($\text{Na}^+ + \text{K}^+$)ATPase], which is responsible for the active transport of Na^+ and K^+ , is distributed universally among animal cell membranes and consists of two types of subunits, α and β (refs 1-4). The larger α -subunit with a relative molecular mass (M_r) of 84,000-120,000 is thought to have the catalytic role. We have now cloned and sequenced DNA complementary to the *Torpedo californica* electroplax messenger RNA encoding the α -subunit of ($\text{Na}^+ + \text{K}^+$)ATPase and have deduced the complete amino-acid sequence of the polypeptide. Some structural features of the α -subunit molecule related to the function of this active-transport protein are discussed.

Tryptic peptides from the purified *T. californica* ($\text{Na}^+ + \text{K}^+$)ATPase were isolated, analysed for amino-acid sequence and five peptide sequences determined (Fig. 1). A library of complementary DNA clones derived from poly(A)⁺ RNA from an electric organ of *T. californica* was then screened by hybridization with a mixture of synthetic oligodeoxyribonucleotides, which represented all possible cDNA sequences corresponding to part of the amino-acid sequence determined for one of the tryptic peptides (Fig. 1e). An upstream fragment of the cDNA insert of a clone thus isolated was used as probe to screen the cDNA library again, and a third screening was carried out with an upstream fragment of the cDNA insert of a clone that resulted from the second screening. Details of the cloning are given in the Fig. 2 legend.

Figure 2 shows the 3,365-nucleotide sequence (excluding the poly(dA) tract) of the cDNA encoding the α -subunit of *T. californica* ($\text{Na}^+ + \text{K}^+$)ATPase. Because the mRNA encoding this polypeptide has a size of ~4,000 nucleotides as estimated by blot hybridization analysis (data not shown), a 5'-terminal sequence of ~500 nucleotides is apparently missing from the sequence shown in Fig. 2. All of the five partial amino-acid sequences determined by peptide analysis (Fig. 1) were found to be encoded by the cDNA sequence in the same reading frame (amino-acid residues 228-240, 431-438, 535-550, 671-690 and 1,011-1,022). By using this reading frame, the amino-acid sequence of the cryptic portion of the ($\text{Na}^+ + \text{K}^+$)ATPase α -subunit was deduced (Fig. 2). The translational initiation site was assigned to the methionine codon at nucleotide residues 1-3 because this is the first in-frame ATG triplet that appears downstream of the nonsense codon TAA (nucleotide residues -39 to -37). Codon 1,022 specifying tyrosine is followed by a translational termination codon (TAG). Thus, we conclude that the α -subunit of *T. californica* ($\text{Na}^+ + \text{K}^+$)ATPase is composed of 1,022 amino-acid residues (including the initiating methionine) and has a calculated M_r of 112,424. This polypeptide does

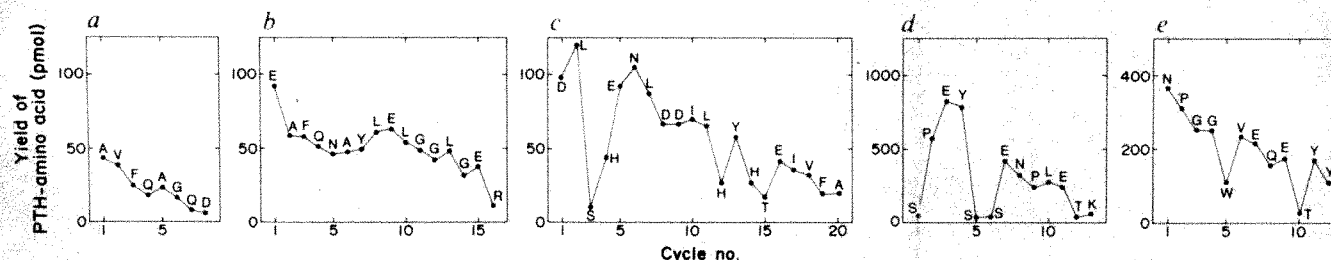
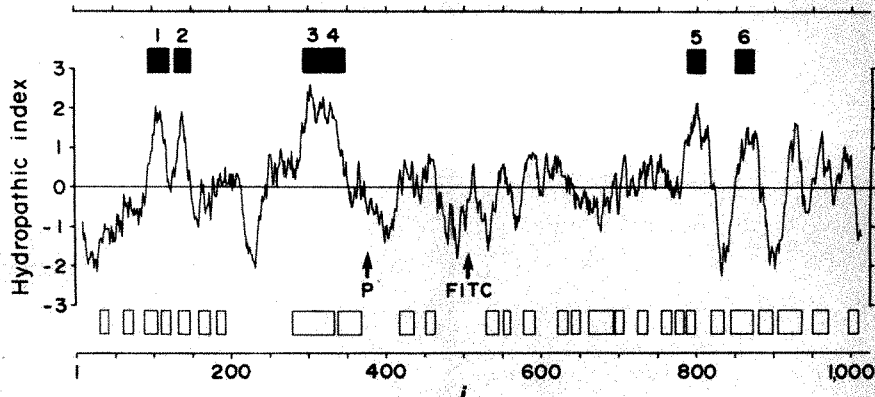


Fig. 1 Sequence analysis of peptides from the α -subunit of *T. californica* ($\text{Na}^+ + \text{K}^+$)ATPase. Fractions Ia (a), IIa (b), IIIa (c), IVa (d) and Va (e) were subjected to amino-acid sequence analysis with a gas-phase sequencer²⁶ (model 470 A, Applied Biosystems).

Methods. The microsomal fraction (673 mg of protein) derived from ~200 g of *T. californica* electric organ (Pacific Bio-Marine Laboratories) was treated with SDS²⁷ in the presence of 3 mM ATP and 30% (w/v) glycerol and centrifuged in the discontinuous gradient of 30% (w/v) and 42% (w/v) sucrose. The material at the interface was collected and treated with 3 mg ml⁻¹ octaethylene glycol mono-*n*-dodecyl ether⁹ at a protein concentration of 1.5 mg ml⁻¹ and then centrifuged. The resulting supernatant contained 81 mg of purified ($\text{Na}^+ + \text{K}^+$)ATPase. The enzyme was treated with 1% SDS and fractionated by gel filtration on a Biogel A-1.5m column²⁸ (2.5 × 100 cm). The fraction consisting mainly of the α -subunit was subjected to chromatography on a concanavalin A-Sepharose column²⁹ (1.2 × 11 cm) followed by gel filtration on the same Biogel A-1.5m column to yield 1.2 mg of purified α -subunit. This was dialysed for two days against 10 mM sodium phosphate buffer, pH 7.2, containing 6 M urea, in which particles of Dowex AG1-X2 resin were suspended for efficient removal of SDS, and was then dialysed for 20 h against 0.1 M ammonium bicarbonate, pH 8.0 or 0.2 M ammonium acetate, pH 8.0. The dialysed material was concentrated and treated with TPCK-treated trypsin (Worthington) at room temperature for 16 h in the absence or presence of 10 mM CaCl₂; the α -subunit/trypsin ratio was 50. A further identical aliquot of TPCK-treated trypsin was added, and the incubation was continued for additional 8 h. The tryptic digest was directly loaded onto a Hi-Pore C₄ column (4.6 × 250 mm; Bio-Rad) equilibrated with 0.1% trifluoroacetic acid, and a linear gradient of 0-50% (over 50 min) or 0-30% (over 60 min) acetonitrile was applied at a flow rate of 1 ml min⁻¹. Five fractions were collected and rechromatographed on the same column to yield fractions Ia-Va. Phenylthiohydantoin (PTH)-amino acids were analysed by HPLC at 40 °C using a Senshu-Pak column (4.6 × 300 mm) with a three-solvent gradient (acetonitrile, water and 40 mM sodium acetate buffer, pH 4.7) at a flow rate of 1 ml min⁻¹. The yield of PTH-amino acids at each cycle of Edman degradation is shown; the one-letter amino acid notation is used.

Methods. The Okayama-Berg cDNA library³² used was the same as that screened for cDNAs encoding the acetylcholine receptor subunits³³. The transformation and screening procedures were as described previously³⁴. The oligodeoxyribonucleotide probe used for isolating pNKA α 1 was a mixture of 32 hexadecamers, 5'-GT₂TC₂TG₂TCNACC-3' (synthesized³⁵ as two pools containing A/T or G/C at position 12, where N denotes a mixture of A, T, G and C), which corresponds to the sequence Trp-Val-Glu-Gln-Glu-Thr at positions 1,015-1,020; the probe was ³²P-labelled at the 5' end. Screening of $\sim 2.5 \times 10^4$ transformants yielded 56 clones that hybridized with the probe at 46°C. Four randomly selected clones were isolated; pNKA α 1 carried apparently the largest cDNA insert among them. To clone a further upstream sequence, we screened the same cDNA library by hybridization at 60°C with the *Bst*NI(2,579)/*Taq*I(2,739) fragment excised from pNKA α 1 and labelled by nick-translation³⁶ with [α -³²P]dCTP; the restriction endonuclease sites are identified by numbers indicating the 5'-terminal nucleotide generated by cleavage. From $\sim 5 \times 10^4$ transformants, 136 hybridization-positive clones were thus obtained. Among the 31 clones selected randomly, pNKA α 8 harboured apparently the largest cDNA insert. The filters screened for isolating pNKA α 8 were re-used for hybridization at 68°C with the nick-translated ~ 250 -base-pair fragment (excised from pNKA α 8) extending from the *Pst*I site flanking the cDNA insert to the *Pst*I(604) site. Among the seven hybridization-positive clones isolated (including pNKA α 8), pNKA α 87 carried apparently the largest cDNA insert.

Fig. 3 Hydropathy profile and predicted secondary structures of the $(\text{Na}^+ + \text{K}^+)\text{ATPase } \alpha$ -subunit. The averaged hydropathic index of a nonadecapeptide composed of amino-acid residues $i-9$ to $i+9$ is plotted against i , where i represents amino acid number; the hydropathy indices of individual amino acids are taken from ref. 12. The positions of the predicted structures of α -helix and/or β -sheet¹³ that have a length of ≥ 10 residues are shown by open boxes, and those of the putative transmembrane segments M1-M6 by closed boxes. The phosphorylation and FITC-reactive sites are indicated.



not possess a hydrophobic amino-terminal sequence characteristic of the signal peptide⁵.

The sequence of amino-acid residues 2-17 (with a gap of residues 4-7) is homologous with the amino-terminal sequence of the mammalian^{6,7} and avian $(\text{Na}^+ + \text{K}^+)\text{ATPase } \alpha$ -subunit⁸, and the sequence of residues 2-47 (with gaps of residues 5-19, 22 and 27-30) with that of the *Artemia salina* counterpart⁹. Furthermore, some of the partial sequences of the lamb kidney $(\text{Na}^+ + \text{K}^+)\text{ATPase } \alpha$ -subunit, determined by analysis of tryptic peptides¹⁰, have homologous counterparts in the *Torpedo* sequence (for example, residues 502-512, 596-606 and 647-657). Five potential *N*-glycosylation sites¹¹ (residues 215, 405, 483, 637 and 719) are found in the *Torpedo* α -subunit sequence, but all of them are assigned to the cytoplasmic side of the membrane (see below).

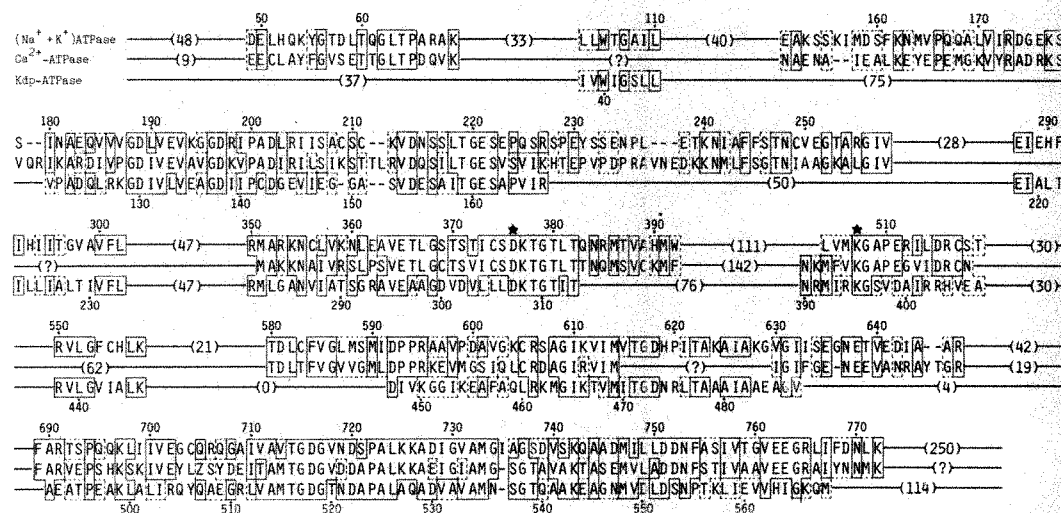
Analysis of the amino-acid sequence of the $(\text{Na}^+ + \text{K}^+)\text{ATPase } \alpha$ -subunit for local hydropathy¹² and predicted secondary structure¹³ (Fig. 3) suggests the presence of at least six transmembrane segments forming presumably α -helical structures, that is, M1 (residues 95-122), M2 (129-150), M3 (294-318), M4 (320-348), M5 (786-809) and M6 (848-873). These hydrophobic segments comprise a continuous stretch of 22-29 uncharged residues including many nonpolar residues, except that segment M4 contains a glutamic acid residue (residue 334) in the middle, which is flanked by proline and glycine. If segment M5 or M6 is assumed to extend over a wider range (residues 774-823 and 848-885, respectively), the possibility

remains that one or both of these segments span the membrane twice in a hairpin shape. If this was the case, segments M5 and M6 would also contain three and two acidic residues, respectively, as sole charged residues.

It is possible that the transmembrane segments take part in the cation-conducting pathway and that the acidic residues within these segments are involved in the transport of cations. Of interest in this context is that $(\text{Na}^+ + \text{K}^+)\text{ATPase}$, in its normal catalytic cycle, passes through states in which cations are occluded within the enzyme molecule so that they are not exchangeable^{14,15}. Three additional hydrophobic segments comprising exclusively uncharged residues (residues 919-931, 961-973 and 984-1,000) are present near the carboxy-terminus (Fig. 3). They seem to be too short to span the membrane, but the possibility remains that some of them traverse the membrane if a longer stretch is assumed. They may be associated with the membrane or may be involved in hydrophobic inter- or intra-subunit interaction.

The sequence surrounding the aspartic acid residue that is phosphorylated by ATP¹⁶ (Cys-Ser-Asp-Lys) is located at residues 374-377. The site reactive with fluorescein 5'-isothiocyanate (FITC)^{17,18} (His-Leu-Leu-Val-Met-Lys-Gly-Ala-Pro-Glu-Arg), which is thought to be at or near the ATP binding site, is positioned at residues 502-512, except that the amino-terminal histidine residue is replaced by tyrosine. Because these functional regions reside on the cytoplasmic side of the membrane, the transmembrane topology of the $(\text{Na}^+ + \text{K}^+)\text{ATPase}$

Fig. 4 Alignment of the amino-acid sequences of the *T. californica* $(\text{Na}^+ + \text{K}^+)\text{ATPase } \alpha$ -subunit (top), rabbit skeletal sarcolemmal reticulum Ca^{2+} -ATPase (middle) and the KdpB protein of *E. coli* Kdp-ATPase (bottom). The sequences of the Ca^{2+} -ATPase and the KdpB protein have been taken from refs 24 and 25, respectively. The one-letter amino-acid notation is used. Sets of identical residues are enclosed with solid lines and sets of conservative residues by dotted lines; conservative substitutions are defined



as pairs of residues belonging to one of the following groups: S, T, P, A and G; N, D, E, Q and Z; H, R and K; M, I, L and V; F, Y and W (ref. 37). Gaps (-) have been inserted to achieve maximum homology. The non-homologous segments are shown by lines together with the sum of residues present in each segment given in parentheses. The residues of the $(\text{Na}^+ + \text{K}^+)\text{ATPase } \alpha$ -subunit and the KdpB protein are numbered beginning with the initiating methionine of the respective polypeptides. The residues of the Ca^{2+} -ATPase, whose numbers (see ref. 24) are not shown, are as follows (from the amino- to the carboxy-terminus): sequence 1 (S1), 10-30; S2, 3-115; S3, 1-42; S3, 185-201; S3, 264-298; S4, 1-18; S4, 38-120. The aspartic acid residue that is phosphorylated and the lysine residue reactive with FITC are marked with asterisks. For evaluating homology (see text), only the segments comprising residues 180-227, 351-382, 504-518, 592-614, 630-632 and 690-767 of $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ have been taken into account; gaps have been counted as one substitution regardless of their length, and the position including Z has been excluded from the calculation for all pairs.

α -subunit molecule is assigned such that the amino-terminal region, the regions between segments M2 and M3 and between segments M4 and M5 and the carboxy-terminal region are located on the cytoplasmic side (assuming the presence of six transmembrane segments). Thus, the regions between segments M1 and M2, between segments M3 and M4 and between segments M5 and M6 are assigned to the extracellular side of the membrane, where the ouabain binding site is situated. The transmembrane topology assigned is generally consistent with the model proposed on the basis of specific covalent labelling combined with proteolysis¹⁹⁻²¹. Furthermore, our assignment generally supports the asymmetric partition of the α -subunit molecule across the membrane, favouring the cytoplasmic region^{22,23}.

Figure 4 shows the alignment of the homologous amino-acid sequences of the *T. californica* ($\text{Na}^+ + \text{K}^+$)ATPase α -subunit, rabbit skeletal sarcoplasmic reticulum Ca^{2+} -ATPase²⁴ and the KdpB protein of *Escherichia coli* Kdp-ATPase²⁵ (more than half of the sequence of the Ca^{2+} -ATPase has been determined by protein analysis²⁴). The degree of homology for the total segments where sequences of all the three polypeptides can be aligned is 58, 37 and 36% for the pairs ($\text{Na}^+ + \text{K}^+$)-ATPase/ Ca^{2+} -ATPase, ($\text{Na}^+ + \text{K}^+$)ATPase/Kdp-ATPase and Ca^{2+} -ATPase/Kdp-ATPase, respectively. This suggests that the three polypeptides evolved from a common ancestor. The regions conserved in all the three polypeptides, including the phosphorylation and FITC binding sites, may be involved in the basic energy transduction process common to these ion transport ATPases rather than in their ion selectivity, which is different for each enzyme.

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Voltage-dependent ATP-sensitive potassium channels of skeletal muscle membrane

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It has been known for some years that skeletal muscle develops a high potassium permeability in conditions that produce rigor, where ATP concentrations are low and intracellular Ca^{2+} is high¹. It has seemed natural to attribute this high permeability to K channels that are opened by internal Ca^{2+} , especially as the presence of such channels has been demonstrated in myotubes and in the transverse tubular membrane system of adult skeletal muscle²⁻⁴. However, as we show here, the surface membrane of frog muscle contains potassium channels that open at low internal concentrations of ATP (<2 mM). ATP induces closing of these channels without being split, apparently holding the channels in one of a number of closed states. The channels have at least two open states whose dwell times are voltage-dependent. Surprisingly, we find that these may be the most common K channels of the surface membrane of skeletal muscle.

To record unitary currents through the potassium channels, we have used the patch-clamp technique⁵ on large sarcolemmal vesicles prepared by KCl loading and enzymatic treatment^{6,7}. We used recording pipettes filled with a 60 mM KCl solution in most cases, and held the membrane potential of the patch at a negative level. In these conditions, channel activity appeared only when the patch was excised from the vesicle, so that its inner surface came into contact with a 120 mM KCl solution, free of ATP. Figure 1 illustrates one such experiment, in which the patch was held at a membrane potential of -60 mV and was exposed in turn to solutions containing ATP at 0.5 and 2 mM, interspersed with exposures to ATP-free solution. The presence of ATP reduces the probability of the channels being open, P_{open} , from 0.496 ± 0.152 ($n = 16$) in ATP-free solution at -60 mV (60 mM external $[\text{K}]_0$) to 0.154 ± 0.033 ($n = 4$) in 0.5 mM ATP.

ATP closes channels without being metabolized. First, most of the patches were exposed to internal solutions free of Mg^{2+} (three of the four patches exposed to 0.5 mM ATP). Second, the non-metabolizable analogue of ATP, adenylylimidodiphosphate (AMP-PNP, 0.5 mM) was equally effective at closing channels, reducing P_{open} to 0.129 and 0.226 in two patches. (In one further patch, 0.5 mM ATP and 0.5 mM AMP-PNP both silenced all four channels that the patch contained.) However, ADP was much less effective than ATP.

The ATP-sensitive channel is selective for K^+ , as its current-voltage relation (Fig. 2c) reverses at the equilibrium potential for potassium ions (E_K). With 60 mM K^+ in the pipette, this reversal potential was -18.2 ± 0.7 mV ($n = 5$), close to the theoretical value of -17.3 mV. Reducing the pipette K^+ concentration altered the reversal potential appropriately, to -27 mV in 40 mM $[\text{K}]_0$ (one patch) and to -54 and -60 mV (two patches) in 10 mM $[\text{K}]_0$. Channel conductance was also dependent on external K^+ concentration, being reduced from 48.2 ± 1.5 pS ($n = 5$) in 60 mM K^+ to 17.8 and 18.0 pS in 10 mM K^+ . The channel is highly selective: Rb^+ does not permeate when it is substituted for K^+ in the pipette (not shown), even though it permeates several other classes of K channel.

As well as being decreased by the presence of ATP, channel opening is also affected by membrane potential, although it is not increased by Ca^{2+} , unlike the opening of Ca-activated K channels, which also have a higher unitary conductance³. We have demonstrated the voltage dependence in two ways: by holding the potential close to E_K and then stepping the potential for periods of 50 ms (Fig. 2a, b), and by recording for prolonged periods (average ~90s) at different holding potentials and subsequently measuring open and closed times (Fig. 3). The experi-

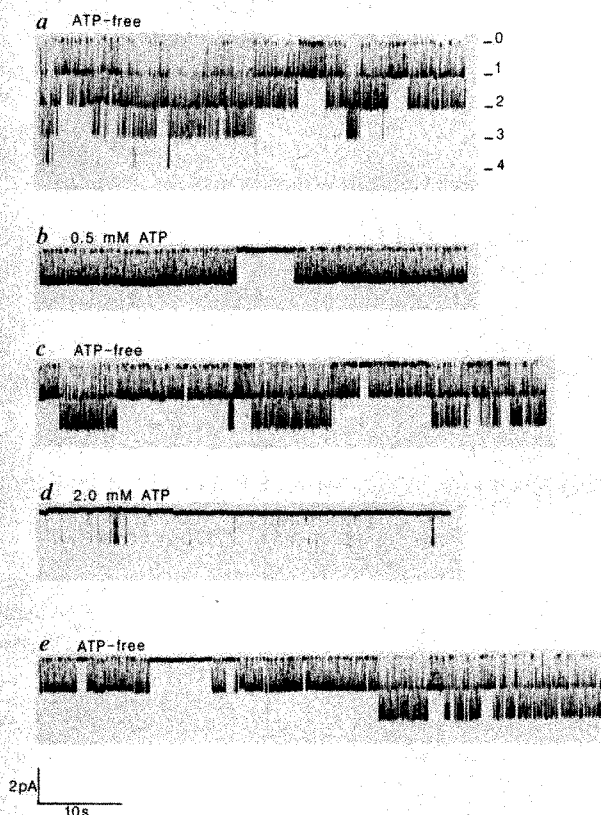


Fig. 1 Effect of ATP on K^+ -channel currents in an inside-out patch of membrane excised from a sarcolemmal vesicle formed from frog muscle⁷. The patch pipette contained 60 mM KCl, 60 mM NaCl, 1.8 mM $CaCl_2$ and 5 mM Tris-maleate, pH 7.2. The membrane potential was -60 mV throughout. The cytoplasmic face of the membrane was exposed to different ATP concentrations by moving the pipette tip between the mouths of a series of pipes down which test solutions flowed at $1-2$ ml h^{-1} (ref. 16). These solutions contained 5 mM EGTA neutralized with KOH, KCl to bring $[K^+]$ to 120 mM, 2 mM $MgCl_2$, ATP at the concentration indicated, and 10 mM HEPES buffer, pH adjusted to 7.2 with KOH. The patch was exposed to the solutions in the order *a*, *b*, *c*, *d*, *e* and we have shown the total time of recording in each case. Currents were recorded with a List electronic EPC-5 patch-clamp amplifier onto FM tape (Racal Store 4, 30 i.p.s.) and were filtered at 0.5 kHz (3 dB down, 8-pole Bessel) before display. The current levels corresponding to 0, 1, 2, 3 and 4 open channels are indicated at the right of record *a*. P_{open} was calculated as $(\sum_{j=1}^N t_j)/TN$ where t_j is the time spent at each level, j corresponding to 1, 2, ..., N channels open (where N is the maximum number of channels seen in ATP-free solution), and T is the duration of the recording. Analysis was done using a computer after the current record had been digitized (see Fig. 2 legend). In this experiment the P_{open} values were: *a*, 0.401; *b*, 0.175; *c*, 0.251; *d*, 0.004; *e*, 0.222. Mean values in ATP-free solution and in 0.5 mM ATP are given in the text. A second patch exposed to 2 mM ATP had $P_{open} = 0.012$. In two patches exposed to 0.1 mM ATP, P_{open} was 0.255 and 0.225; in the same patches in 0.02 mM ATP, P_{open} was 0.398 and 0.344.

ments involving stepping of the membrane potential show that the mean current per trace declines with time under hyperpolarization (Fig. 2*a*), indicating that hyperpolarization partly inactivates the potassium conductance. However, depolarization from -17 mV does not further activate conductance (Fig. 2*b*). We used this stepping procedure routinely to measure current-voltage relations for the channel (as in Fig. 2*c*).

The records of Figs 1 and 2*a* indicate that the channel opens and shuts rapidly. Channel openings occur in bursts separated by intervals that may be quite brief (Fig. 2*a*) or very long (Fig. 1*b*, *e*), showing that the channel has complex kinetics. Open-time histograms (Fig. 3*a-c*) are better fitted by two exponentials, suggesting two open states⁸. The presence of two components in the open time distribution is clearer at -40 mV (Fig. 3*c*) than at -60 mV (Fig. 3*a*), partly because of an increase in time

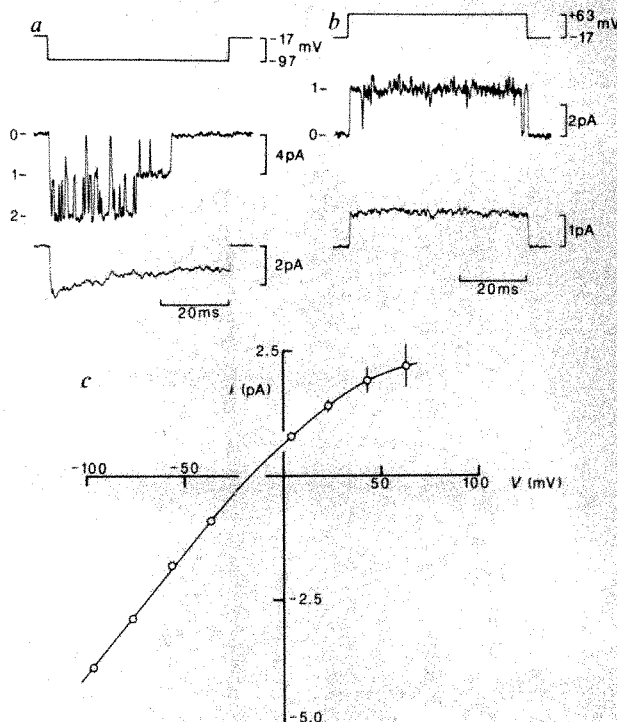


Fig. 2 *a*, Single-channel currents recorded from ATP-sensitive channels when a membrane patch was stepped for 50 ms from the holding potential of -17 mV (close to the calculated E_K) to -97 mV. The top trace shows the voltage step, the middle trace a sample current record, and the bottom trace the average of 64 such current records. The current was filtered at 2 kHz and digitized at 10 kHz (Cambridge Electronic Design 502) before analysis with a DEC PDP 11/23 computer. Capacitive and leakage currents caused by the voltage step have been removed from the current trace by analog compensation and digital subtraction⁷. For display, the number of data points in the current records was increased fourfold by interpolation using a cubic spline method¹⁷. We used this voltage-step protocol as it has been commonly used to study the 'inward rectifier' K channels of muscle¹⁸. The ATP-sensitive channels described here are not inward rectifier channels, however, as they are not open at the resting potential on intact muscle fibres and they show only a slight rectification, (*c*), rather than the extreme rectification associated with the inward rectifier. Also, their single-channel conductance is much larger than that measured for inward rectifier channels by noise analysis¹⁹. *b*, Currents recorded as in *a* but for a step in potential to $+63$ mV. Top trace, voltage; middle trace, current; bottom trace, average of 24 current records. *c*, Mean single-channel current-voltage ($I-V$) relation for the ATP-sensitive channel. Single-channel currents were recorded using 50-ms steps in membrane potential as in *a* and *b*; current amplitude was measured by fitting gaussian distributions to histograms of digitized current values⁹. The points show the mean values for four or five patches and the bars show the s.e.m. where this is larger than the symbols. The recording pipette contained 60 mM K^+ , while the solution bathing the cytoplasmic face of the patch contained 120 mM K^+ . The decrease in conductance at positive potentials was associated with an increase in the open-channel current noise, as can be seen in the middle record of *b*.

constant of the slower component with depolarization. There appear to be relatively more brief openings in the presence of ATP (Figs 1*d*, 3*b*).

Closed-time distributions (Fig. 3*d-f*) indicate the presence of at least three closed states. However, we have not recorded for sufficiently long periods to measure the length of more than a few of the very long periods of closing, which are therefore not well represented in the closed-time histograms of Fig. 3. In fact, ATP apparently increases the chance of the channel entering a closed state with a very long dwell time. ATP reduced the several levels of opening seen in multichannel patches to one or two levels of opening (see Fig. 1), indicating that several of the channels are silent throughout the 90-s recording period. The shorter closed times in Fig. 3*b* are little affected by ATP.

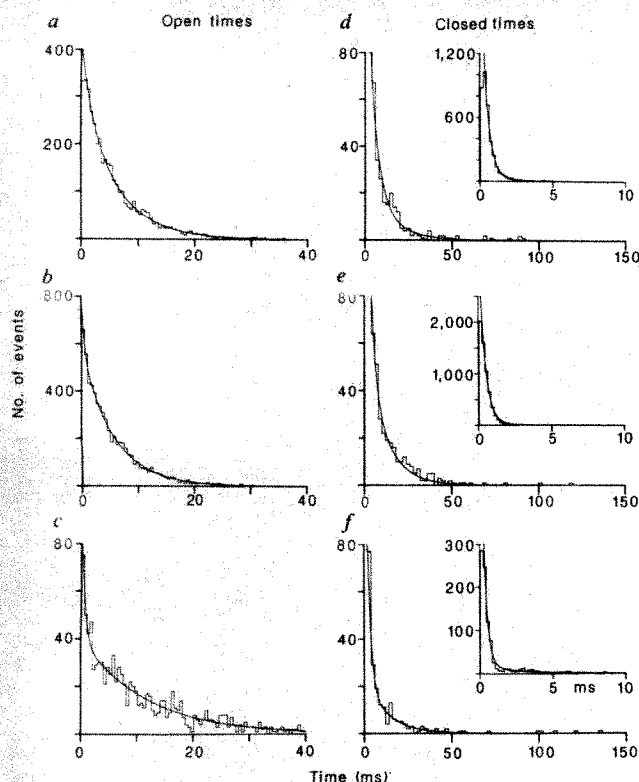


Fig. 3 Histograms of open (*a-c*) and closed (*d-f*) times for the ATP-sensitive channel, constructed from periods of recording lasting between 39 and 51 s, during which only a single level of opening was seen. The pipette contained 60 mM K⁺. *a-c*, Effects of ATP (*a, b*) and membrane potential (*a, c*) on open-time distribution. These distributions have been fitted using a least-squares algorithm to the double-exponential probability density function (p.d.f.):

$$f(t) = A \exp(-t/\tau_1) + B \exp(-t/\tau_2).$$

ATP increases the value of *A*, reduces τ_1 , but has little effect on τ_2 . Depolarization increases τ_2 . *a*, Open-time histogram in ATP-free solution; holding potential -60 mV. The fitted p.d.f. has $A = 0.029 \text{ ms}^{-1}$, $B = 0.168 \text{ ms}^{-1}$, $\tau_1 = 1.85 \text{ ms}$ and $\tau_2 = 5.65 \text{ ms}$. *b*, Open-time histogram at -60 mV in 0.5 mM ATP solution for the same patch as *a*. The fitted p.d.f. has $A = 0.109 \text{ ms}^{-1}$, $B = 0.172 \text{ ms}^{-1}$, $\tau_1 = 0.34 \text{ ms}$, $\tau_2 = 5.58 \text{ ms}$. *c*, Open-time histogram at -40 mV in ATP-free solution. The fitted p.d.f. has $A = 0.118 \text{ ms}^{-1}$, $B = 0.081 \text{ ms}^{-1}$, $\tau_1 = 0.59 \text{ ms}$, $\tau_2 = 11.56 \text{ ms}$. *d-f*, Effects of ATP (*d, e*) and membrane potential (*d, f*) on closed-time distribution. These distributions have been fitted with three exponentials. The two slowest were fitted to a histogram formed with 2-ms bins and the fastest was fitted after the histogram had been re-formed using 0.2-ms bins (insets). The fitted p.d.f. is given by

$$f(t) = A \exp(-t/\tau_1) + B \exp(-t/\tau_2) + C \exp(-t/\tau_3)$$

ATP has little effect on the distribution measured, but appears to place channels in a closed state too long-lived to contribute significantly to the histograms shown. *d*, Closed-time histograms in the same conditions and for the same patch as in *a*. The fitted p.d.f. has $A = 2.231 \text{ ms}^{-1}$, $B = 0.012 \text{ ms}^{-1}$, $C = 0.002 \text{ ms}^{-1}$, $\tau_1 = 0.41 \text{ ms}$, $\tau_2 = 4.56 \text{ ms}$, $\tau_3 = 14.23 \text{ ms}$. *e*, Closed-time histograms in the same conditions as in *b*. The fitted p.d.f. has $A = 2.331 \text{ ms}^{-1}$, $B = 0.011 \text{ ms}^{-1}$, $C = 0.004 \text{ ms}^{-1}$, $\tau_1 = 0.40 \text{ ms}$, $\tau_2 = 2.64 \text{ ms}$, $\tau_3 = 11.07 \text{ ms}$. *f*, Closed-time histograms in the same conditions and for the same patch as in *c*. The fitted p.d.f. has $A = 2.997 \text{ ms}^{-1}$, $B = 0.066 \text{ ms}^{-1}$, $C = 0.008 \text{ ms}^{-1}$, $\tau_1 = 0.26 \text{ ms}$, $\tau_2 = 1.8 \text{ ms}$, $\tau_3 = 12.41 \text{ ms}$.

The ATP-sensitive channels are very common in skeletal muscle. The patches that we have analysed in detail contained an average of 3.6 ± 0.4 channels ($n = 16$). Several other patches were not analysed because of the very high number of channels (occasionally as many as 10 or more) that were active. As our patch electrodes had tips with openings of only 0.3–0.4 μm diameter (as measured with a scanning electron microscope), a high channel density is indicated—as high as or higher than that of ‘delayed rectifier’ K channels in the same preparation⁹.

However, while the role of delayed rectifier channels is well

known (to repolarize the action potential), that of ATP-sensitive channels is less clear, particularly since the ATP concentration in frog muscle is high (4.9 mM)^{10,11} and is well buffered by creatine phosphate and creatine kinase. We find that the channels are closed in patches formed on resting intact muscle, but anticipate that channels will open under rigor conditions, contributing to the high potassium permeability then found¹. Nevertheless, it seems likely that channels that are present at such high density will have an important physiological role. While we point out that the regulating effect of ATP occurs at concentrations much closer to the physiological range than has been reported previously for ATP-sensitive channels of heart muscle¹² and of B cells of pancreatic islets^{13,14}, it is possible that the sensitivity to ATP in intact membranes is different from that found in excised patches or that some other metabolic constituent of sarcoplasm contributes to their control.

In man, the severest exercise may reduce the concentration of ATP in muscle by 45% (ref. 15). As we have found that open times for the ATP-sensitive channel also increase with depolarization, the channel may play a part in the regulation of K conductance during muscle activity. Alternatively, as the channel is present in surface membrane of muscle (and in non-contractile cells^{13,14}), its role may be primarily to sense submembrane, rather than myofibrillar, levels of ATP or other metabolites.

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I–J as an idio type of the recognition component of antigen-specific suppressor T-cell factor

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The I–J determinant of membrane glycoprotein is known to be expressed exclusively on suppressor T cells (Ts), which have a crucial role in the regulation of immune responses¹. I–J also comprises part of the soluble factor (TsF) with suppressor activity which is secreted from Ts. Gene-mapping experiments have indicated that the I–J gene lies between the I–A and I–E subregions of the mouse major histocompatibility complex (MHC)^{2,3} and is defined by the H-2 congenic pair, that is, B10.A(3R) and B10.A(5R)⁴. In fact, antibodies raised in the reciprocal combina-

tions of B10.A(3R) and B10.A(5R) define the $I-J^b$ and $I-J^k$ alleles, and are able to detect the I-J determinants on Ts and TsF. Biochemical and functional analyses⁵⁻¹⁰, using I-J-positive Ts clones and hybridomas, have demonstrated that monoclonal anti-I-J antibodies precipitate $I-J^k$ or $I-J^b$ with a relative molecular mass of 25,000–28,000 (25–28K) and that the $I-J^+$ molecule mediates the restriction specificity of TsF in association with an antigen-binding protein (45K). However, molecular genetic studies on the $I-J$ gene¹¹⁻¹³ reveal no genetic difference between B10.A(3R) and B10.A(5R) and also that there is no room to accommodate a gene encoding I-J in the expected I region. These discrepancies between the molecular genetic and serological/functional data require explanation. Here we demonstrate that Ts and TsF expressing I-J of the host type were produced by fully allogeneic bone marrow cells of donor origin in chimaeric mice, when the chimaeras received the host antigen-presenting cells (APC) at the time of immunization. The results show that APC are necessary for the activation and clonal expansion of Ts and also support the notion that I-J is an idiotypic determinant of the recognition component of Ts and TsF.

To analyse the nature of I-J, chimaeric mice were prepared as described elsewhere¹⁴. C57BL/6($H-2^b$) or C3H($H-2^k$) mice were irradiated with 10.63 Gy and reconstituted with T-cell-depleted allogeneic bone marrow cells from C3H($H-2^k$), C57BL/6($H-2^b$), B10.A(3R)($H-2^{13}$) or B10.A(5R)($H-2^{15}$) mice. The chimaeras were kept for 4 months, then immunized with keyhole limpet haemocyanin (KLH). Primed thymocytes and spleen cells were tested for their H-2 phenotypes before preparation of TsF and were shown to possess the H-2 type of donor origin (Table 1). KLH-specific suppressor T-cell factor (KLH-TsF) was prepared by freezing and thawing of thymocytes from KLH-primed chimaeric mice, as described elsewhere³, and tested for its effects on the *in vitro* secondary anti-dinitrophenyl (DNP) IgG plaque-forming cell (PFC) responses of $H-2^k$ and $H-2^b$ mice. As shown in Table 2, KLH-TsF from the C3H $\rightarrow$ C57BL/6 chimaera, like C3H TsF, suppressed the C3H but not the C57BL/6 response in a KLH-specific manner, because only the C3H response to DNP-KLH, but not to DNP-ovalbumin (OVA), was suppressed. Similarly, the C57BL/6 $\rightarrow$ C3H TsF specifically suppressed the response of C57BL/6 but not that of C3H. Therefore, KLH-TsF from chimaeric mice expresses the restricting specificity of the donor type. These results indicate that the chimaeric mice produce TsF bearing I-J of the donor type, as the restriction specificity is mediated by the $I-J^+$ molecule^{7,8} and because of the fact that the I-J phenotype of TsF must be the same as that of the responding cells^{7,8,15}. A similar conclusion was reached for chimaeras using B10.A(3R) or B10.A(5R) as the donor (Table 2). B10.A(3R) $\rightarrow$ C3H and B10.A(3R) $\rightarrow$ C57BL/6 produced $H-2^b$ -restricted TsF whereas TsF from either B10.A(5R) $\rightarrow$ C3H or B10.A(5R) $\rightarrow$ C57BL/6 suppressed only the $H-2^k$ responses. Thus, KLH-TsF from chimaeras using B10.A(3R) or B10.A(5R) is the same as KLH-TsF from conventional B10.A(3R) or B10.A(5R) mice, which produce $I-J^b$ -TsF or $I-J^k$ -TsF, respectively⁷.

The results in Table 2 show that the host-specific restriction specificity of TsF is not acquired in the chimaera situation and indicate that the I-J phenotype cannot be changed, thus I-J seems to be an MHC-controlled product. It is, however, possible that only the donor-restricted but not host-specific Ts are allowed to expand after antigenic stimulation because there is no APC of the host type present and most APC are replaced by those of donor origin in the 4-month chimaera^{14,16}. To test this possibility, the chimaeric mice were given APC of the host type at the time of immunization with KLH. As shown in Table 2, KLH-TsF from either the C3H $\rightarrow$ C57BL/6 or C57BL/6 $\rightarrow$ C3H chimaera that received the host APC specifically suppressed the responses of both C57BL/6 and C3H but not those of a third party, such as BALB/c mice, despite the fact that these chimaeric thymocytes expressed H-2 of donor type alone. It is clear, therefore, that fully allogeneic chimaeras (subjected to irradiation of the bone marrow) can generate both donor and host H-2-restricted KLH-TsF. Whether the former or the latter type of TsF is

Table 1 H-2 typing of thymocytes from fully allogeneic chimaeras

Chimaeras	% Cytotoxicity with antibodies against			
	H-2D ^b	H-2D ^d	H-2K ^b	H-2K ^k
C3H $\rightarrow$ C57BL/6	<10%	—	—	>95%
C57BL/6 $\rightarrow$ C3H	>95%	—	—	<10%
C3H $\rightarrow$ C57BL/6 with C57BL/6 APC	<10%	—	—	>95%
C57BL/6 $\rightarrow$ C3H with C3H APC	>95%	—	—	<10%
B10.A(3R) $\rightarrow$ C57BL/6	<10%	>95%	—	—
B10.A(3R) $\rightarrow$ C3H	—	—	>95%	<10%
B10.A(5R) $\rightarrow$ C57BL/6	<10%	>95%	—	—
B10.A(5R) $\rightarrow$ C3H	—	—	>95%	<10%

Fully allogeneic chimaeric mice were prepared as described previously¹⁴. Briefly, C57BL/6($H-2^b$) or C3H($H-2^k$) mice were irradiated with 10.63 Gy of ¹³⁷Cs γ -rays, and reconstituted 24 h later with 10⁷ T cell-depleted allogeneic bone marrow cells from C3H($H-2^k$), C57BL/6($H-2^b$), B10.A(3R)($H-2^{13}$) or B10.A(5R)($H-2^{15}$) pretreated with monoclonal anti-Thy-1.2 antibody (F7D5; Olac Ltd, Bicester, UK) plus complement. The mice were maintained for 4 months in pathogen-free conditions and then used for experiments. The chimaeras (with or without addition of APC of the host type) were immunized with 200 μ g of keyhole limpet haemocyanin (KLH) after a 2-week interval as described elsewhere³. Primed spleen cells and thymocytes from the chimaeras were tested for their H-2 phenotypes in a dye exclusion cytotoxicity assay using anti-H-2D^d antiserum (B10A.(2R) anti-B10.A) and various monoclonal anti-H-2 antibodies, that is anti-H-2D^b (H141-30; BALB/c anti-C57BL/6), anti-H-2K^b (K9-178.9; (C3H $\times$ A)F₁ anti-70.Z/3) and anti-H-2K^k (H100-5.28, BALB/c anti-CBA) (given by Dr G. J. Hammerling) before preparation of TsF. For the cytotoxicity test, spleen cells or thymocytes (10⁵) from the chimaeras were resuspended in Dulbecco's modified minimal essential medium enriched with 2% fetal calf serum (FCS) and were incubated with 20 μ g of a 1:10 dilution of anti-H-2 antiserum and monoclonal anti-H-2 antibodies at a final concentration of 1:1,000 for 15 min at room temperature. The cells were washed twice, then reacted with 20 μ l of a 1:10 dilution of rabbit complement at 37 °C for 30 min. Dead and viable cells were counted under the light microscope by Trypan blue dye exclusion.

generated depends solely on the H-2 type of the APC available at the time of antigen stimulation. The above results also show that APC are necessary for the activation and clonal expansion of Ts. These findings support the data of Takaoki *et al.*¹⁷ and Lowy *et al.*¹⁸, who demonstrated that accessory cells are involved at various stages in suppressor cell interactions.

Two further experiments were done to obtain direct evidence that I-J of the host type is indeed generated from the donor cell type. The first experiment was designed to investigate the H-2 phenotype of the cells producing TsF having host or donor restriction specificity. Thymocytes from C57BL/6 $\rightarrow$ C3H chimaeras that received C3H APC were treated with antibodies against H-2 products of the host or donor type, together with complement, before preparing the chimaeric TsF. The TsF was then tested for its activity. The results are shown as Expt. 3 of Table 2. The chimaeric TsF suppressed both C57BL/6 and C3H responses. However, TsF obtained from the chimaeric thymocytes pretreated with antibody against H-2 of donor type failed to suppress the responses, whereas both B6 and C3H responses were suppressed by TsF from chimaeric thymocytes pretreated with anti-host antibody and complement. Therefore, both donor- and host-restricted TsF were produced by the cells bearing H-2 of the donor type.

In the second experiment, KLH-TsF derived from the chimaeric mice (either C3H $\rightarrow$ C57BL/6 or C57BL/6 $\rightarrow$ C3H) that received host APC was applied to a column of anti-I-J^b or anti-I-J^k. The effluent and the acid eluate were tested for their suppressor activity on the C57BL/6 or C3H response. Table 3 shows that KLH-TsF from the C3H $\rightarrow$ C57BL/6 chimaera that received C57BL/6 APC suppressed the responses of both C3H and C57BL/6. However, after adsorption to the anti-I-J^b column, the chimaeric TsF lost its suppressor effect on the $H-2^b$ response. The $H-2^b$ -restricted suppressor activity was recovered in the eluate from the column. The opposite results were

Table 2 Genetically restricted suppression by KLH-TsF obtained from chimaeric mice

Origin of KLH-TsF	Anti-DNP IgG PFC per 10 ⁶ cultured cells			DNP-OVA	
	DNP-KLH			C57BL/6	C3H
Expt 1	C57BL/6	C3H	BALB/c	C57BL/6	C3H
—	4,510 ± 370	2,210 ± 190	2,440 ± 240	840 ± 40	790 ± 80
C3H	3,910 ± 230	210 ± 170	2,250 ± 260	990 ± 200	750 ± 20
C3H → C57BL/6	4,690 ± 670	730 ± 240	2,290 ± 240	1,010 ± 110	830 ± 110
C3H → C57BL/6 + C57BL/6 APC	920 ± 200	610 ± 190	2,450 ± 370	910 ± 110	760 ± 70
C57BL/6	800 ± 210	2,430 ± 440	2,370 ± 290	1,010 ± 90	870 ± 100
C57BL/6 → C3H	880 ± 270	2,290 ± 360	2,050 ± 240	910 ± 170	820 ± 20
C57BL/6 → C3H + C3H APC	1,160 ± 400	590 ± 60	2,530 ± 290	970 ± 120	810 ± 110
BALB/c	3,990 ± 210	2,360 ± 250	440 ± 70	ND*	ND
Expt 2	C57BL/6	C3H	BALB/c	C57BL/6	C3H
—	4,550 ± 510	1,430 ± 80	ND	700 ± 70	490 ± 100
B10.A(3R)	490 ± 130	1,510 ± 150	ND	720 ± 100	430 ± 80
B10.A(3R) → C57BL/6	1,090 ± 290	1,450 ± 250	ND	660 ± 100	470 ± 40
B10.A(3R) → C3H	1,340 ± 150	1,370 ± 250	ND	670 ± 40	430 ± 70
B10.A(5R)	4,160 ± 170	290 ± 80	ND	750 ± 50	450 ± 60
B10.A(5R) → C57BL/6	3,540 ± 480	500 ± 70	ND	670 ± 130	470 ± 40
B10.A(5R) → C3H	4,150 ± 270	500 ± 140	ND	660 ± 60	450 ± 120
Expt 3	C57BL/6	C3H	BALB/c	C57BL/6	C3H
—	4,120 ± 450	1,970 ± 220	3,670 ± 430	1,080 ± 80	1,000 ± 80
C57BL/6	800 ± 180	1,960 ± 260	3,890 ± 60	1,050 ± 60	1,140 ± 100
C3H	4,050 ± 190	410 ± 50	4,120 ± 100	1,150 ± 60	1,090 ± 50
C57BL/6 → C3H + C3H APC	1,000 ± 140	280 ± 40	3,850 ± 110	1,120 ± 70	1,070 ± 180
C57BL/6 → C3H + C3H APC treated with C	4,030 ± 430	2,050 ± 160	3,930 ± 280	1,160 ± 40	1,010 ± 190
C57BL/6 → C3H + C3H APC treated with anti-H-2D ^b + C	1,010 ± 140	400 ± 80	3,830 ± 360	1,070 ± 190	990 ± 150
C57BL/6 → C3H + C3H APC treated with anti-H-2K ^b + C	3,880 ± 180	1,760 ± 250	360 ± 430	1,120 ± 80	1,000 ± 40

KLH-specific TsF (KLH-TsF) was prepared by freezing and thawing of thymocytes from chimaeric or non-chimaeric mice that had been prime with 200 µg of KLH twice at a 2-week interval as described elsewhere³. In some experiments, the chimaeric mice were injected intravenously twice with 2 × 10⁶ APC (anti-Thy-1-treated peritoneal exudate cells) of the host type at the same time as immunization with KLH. Peritoneal exudate cells were prepared according to the method described elsewhere²³, and treated with anti-Thy-1 and complement (C). The phenotypes of peritoneal exudate cells used were determined in a cytotoxicity assay, and over 95% were found to be Ia-positive cells, with no T-cell contamination (n Thy-1⁺, Lyt-1⁺ or Lyt-2⁺). Two weeks after the second immunization with KLH, the thymocytes derived from chimaeric mice were used to prepare KLH-TsF. In experiment 3, KLH-primed thymocytes (1 × 10⁷ ml⁻¹) derived from chimaeras that received APC of the host type, were treated with anti-H-2D^b (anti-donor) or anti-H-2K^b (anti-host) antibody plus complement, washed, then subjected to freezing and thawing for TsF preparation. KLH-TsF (25 µl, a concentration equivalent to 1 × 10⁷ thymocytes ml⁻¹) was added at the start of cultivation to spleen cell cultures (1.6 × 10⁶ per well) of C57BL/6 or C3H mice primed 4 weeks previously with 100 µg DNP-KLH or 0.1 µg ml⁻¹ DNP-OVA plus 10⁹ *Bordetella pertussis* vaccine. The cells were cultured for 5 days in a 48-well flat-bottomed plate (Costar 3548) with 0.4 ml of RPMI-1640 medium enriched with 10% FCS in the presence of 0.01 µg ml⁻¹ DNP-KLH or 0.1 µg ml⁻¹ DNP-OVA. Anti-DNP IgG PFC were enumerated by using DNP-coupled sheep erythrocyte. Numbers are the mean (±s.d.) PFC number for three cultures. ND, not determined.

Table 3 Existence of two distinct TsF bearing I-J^b and I-J^k determinants in chimaeric TsF

Origin of KLH-TsF	Column	Materials added	Anti-DNP IgG PFC response in	
			C57BL/6	C3H
—	—	—	3,150 ± 190	1,330 ± 120
C3H → B6 + B6 APC	—	Unadsorbed	530 ± 240	190 ± 140
C3H → B6 + B6 APC	Anti-I-J ^b	Effluent	3,110 ± 150	170 ± 90
C3H → B6 + B6 APC	Anti-I-J ^b	Eluate	730 ± 400	1,330 ± 100
C3H → B6 + B6 APC	Anti-I-J ^k	Effluent	840 ± 340	1,370 ± 180
C3H → B6 + B6 APC	Anti-I-J ^k	Eluate	3,430 ± 400	230 ± 60
B6 → C3H + C3H APC	—	Unadsorbed	670 ± 200	360 ± 110
B6 → C3H + C3H APC	Anti-I-J ^b	Effluent	3,170 ± 300	390 ± 120
B6 → C3H + C3H APC	Anti-I-J ^b	Eluate	400 ± 80	1,470 ± 180
B6 → C3H + C3H APC	Anti-I-J ^k	Effluent	210 ± 200	1,440 ± 70
B6 → C3H + C3H APC	Anti-I-J ^k	Eluate	3,160 ± 390	470 ± 160

KLH-TsF (100 µl, equivalent to 1.5 × 10⁷ thymocytes) from chimaeric mice that received APC of the host type (same materials as used in Table 2) was applied to and incubated with immunoadsorbent columns of anti-I-J^b or anti-I-J^k antiserum for 1 h at 4 °C. The effluent or the acid eluate (0.175 M glycine-HCl buffer, pH 3.2) was dialysed, concentrated to a final volume of 100 µl with Sephadex powder, added to a culture of DNP-KLH-primed spleen cells of C57BL/6(B6)(H-2^b) and C3H(H-2^k) mice, and tested for its suppressor activity (see Table 2). Conventions: anti-I-J antisera were used in this experiment to detect any types of I-J determinants generated in the chimaera. Values are mean number of PFC (±s.d.) for three cultures, expressed as number per 10⁶ cultured cells.

obtained when the same KLH-TsF was applied to an anti-I-J^k column. The H-2^k-restricted TsF was adsorbed to and eluted from the anti-I-J^k column, whereas the H-2^b-restricted TsF was recovered in the effluent. Similar results were obtained for KLH-TsF from the C57BL/6 → C3H chimaera that received C3H APC: the H-2^b-restricted KLH-TsF was recovered in the effluent while the H-2^k-restricted KLH-TsF was obtained in the eluate from the anti-I-J^k column. These results demonstrate that the chimaeric TsF contains two types of TsF bearing I-J of host or donor type, that is, I-J^b-TsF or I-J^k-TsF. Hence, both donor- and host-restricted Ts (anti-H-2^k and anti-H-2^b T cells), each of which produce I-J^k- or I-J^b-TsF, are generated from C3H(H-2^k) bone marrow cells under the influence of H-2^b. C57BL/6(H-2^b) bone marrow cells also produce I-J^k-TsF in the H-2^k environment, implying that I-J of the host type is generated in the chimaera. It is probable, therefore, that I-J represents the idiotype determinant on the variable part of the self-receptor of Ts, as speculated by Schrader¹⁹, and recently discussed by Kurata *et al.*²⁰ and Murphy²¹, and also that expression of the I-J phenotype on KLH-TsF is dictated by the host environment.

If the I-J is the idiotype determinant of the recognition component on TsF, several questions must be answered. First, why does the I-J on TsF or Ts receptor map in the H-2 complex? There is no definite answer to this question as yet. However, the experiments using chimaeras described in Table 2 suggest the existence of a new H-2-encoded class II-like molecule on APC. This molecule seems to be essential for the generation and clonal expansion of I-J⁺ Ts and also necessary for expression of the I-J phenotype. The molecule is apparently expressed on APC at least (Tables 2, 3), but does not seem to be identical to the known Ia molecule (that is I-A or I-E). In fact, the B10.A(5R) → C57BL/6 chimaera produces only the H-2^k-restricted TsF(I-J^k-TsF) even though B10.A(5R) and C57BL/6 mice have the I-A^b subregion in common, and I-A^b APC should be present in the chimaera (Table 2). Involvement of the I-E molecule is also excluded, because the H-2^b mice do express the I-J^b phenotype, although they are unable to express the I-E molecule²².

Second, why do Ts and TsF express I-J with H-2-haplotype specificity? If the I-J idotype is expressed on the self-recognizing structure (the I-J⁺ molecule), the I-J could be complementary to the MHC-encoded molecule on the APC so that the I-J seems to be a reflection of MHC-encoded molecule. If this is the case, the variable (V) gene repertoire of the self-receptor of Ts should be limited. In any event, the genetic identification and biochemical characterization of the molecules on APC will be important for resolving the I-J paradox.

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I-J epitopes are adaptively acquired by T cells differentiated in the chimaeric condition

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I-J has been defined as a locus mapped in the murine major histocompatibility complex (MHC) which encodes serological markers found primarily on the surface of suppressor T cells (Ts) and soluble suppressor factors (TsF)^{1,2}. Recent studies have, however, revealed that there is no such specialized locus within the MHC at the DNA level^{3,4}. As the existence of I-J determinants at the protein level on functional T cells, T-cell clones and hybridomas has been confirmed by several serological and biochemical studies⁵⁻⁷, this contradiction has raised serious arguments in the immunological community concerning the nature, origin and expression of I-J determinants. We have raised a number of monoclonal antibodies against the polymorphic structure of I-J molecules, and have studied the expression of I-J epitopes on T cells derived from irradiated bone marrow chimaeras in which stem cells of different genotype differentiated into T cells under the foreign host MHC environment. The results, presented here, indicate that I-J epitopes are not primarily determined by the MHC genes of the stem cells themselves, but are adaptively acquired by T cells differentiated in the chimaeric condition according to the environmental MHC phenotype. Thus, the serologically detectable I-J epitopes are found to be associated with inducible T-cell receptors recognizing self class II MHC antigens.

We have previously reported an interesting activity of anti-I-J monoclonal antibody, which was used to determine serologically the allelic forms of I-J molecules on T cells⁸. The essential findings were: (1) anti-I-J^k antibody blocks the syngeneic and allogeneic mixed lymphocyte reactions (MLRs) of H-2^k and H-2^a haplotype strains but not that of their H-2 congenic partners (this was caused by the blocking of responding T cells but not of stimulator cells). (2) In the syngeneic MLR of H-2^{k/b} F₁ T cells stimulated by F₁ or either parental strain, anti-I-J^k antibody inhibited the response to H-2^k but not to H-2^b stimulators, indicating that I-J^k epitopes are expressed only on those F₁ responder T cells recognizing H-2^k stimulator molecules but not on those responding to H-2^b stimulators. (3) The pattern of inhibition of the syngeneic and allogeneic MLRs of T cells from individual H-2^k strains by various anti-I-J^k antibodies was different under the control of as yet undetermined non-MHC background genes. Thus, the I-J epitopes were found to be associated with the MLR recognition sites of T cells, which are not determined solely by the H-2 genes but which are also influenced by unknown non-MHC genes. These results prompted us to examine the expression of I-J on T cells of irrelevant H-2 genotypic origin but differentiated in the chimaeric condition.

To determine the nature of I-J epitopes on chimaeric T cells, we also used another set of monoclonal antibodies reactive with I-region-controlled epitopes uniquely expressed on T but not on B cells⁹. These monoclonal antibodies were produced by immunizing A.TH (H-2^d) mice with I-region-incompatible A.TL (H-2ⁱ) lymphoid cells; they were selected for their specific reactivity with some functional T cells and T-cell hybridomas⁹. The antibodies were unable to react with conventional class II antigens on B cells and macrophages, but were able to precipitate a unique polypeptide of relative molecular mass 33,000 (33 K), which is apparently under the control of the I-A subregion of the H-2 complex, from a T-cell hybridoma producing an antigen-specific augmenting T-cell factor (TaF)¹⁰. The interrelationship between this polypeptide and the α and

Table 1 Inhibition of allogeneic MLR of normal and chimaeric mice by anti-I-J and anti-Iat monoclonal antibodies

Responder Antibody	C3H Δ c.p.m. % Inh	B6 Δ c.p.m. % Inh	B6C3F ₁ Δ c.p.m. % Inh	B6C3F ₁ → C3H Δ c.p.m. % Inh	B6C3F ₁ → B6 Δ c.p.m. % Inh	C3H → B6C3F ₁ Δ c.p.m. % Inh	B6 → B6C3F ₁ Δ c.p.m. % Inh	B6 → C3H Δ c.p.m. % Inh	C3H → B6 Δ c.p.m. % Inh
Complement alone	100,858 0	91,457 0	101,450 0	31,367 0	62,938 0	50,095 0	38,234 0	34,699 0	117,835 0
Anti-I-J 1G8	44,620 52	90,296 1	59,101 42	6,976 78	59,155 6	3,786 93	33,417 13	36,035 -4	121,349 -3
4B11	113,105 -2	100,420 -9	104,912 -3	31,618 0	61,910 1	55,157 -10	39,890 -4	32,222 7	116,697 1
KN34	43,608 61	85,610 6	60,377 40	11,372 64	59,953 5	22,344 59	11,419 70	14,758 57	115,532 2
Anti-Iat 1L9	56,979 49	87,721 4	60,045 41	6,260 80	63,786 -1	27,518 50	35,891 6	34,148 2	123,705 -5
2L2	62,001 44	83,034 9	59,422 41	9,007 71	63,260 0	24,287 55	17,511 54	13,315 62	117,154 1
14P	106,987 3	93,849 -3	96,793 4	29,875 5	59,669 5	53,494 3	40,657 -6	33,671 0	125,444 -6
74L	103,709 6	91,894 -1	94,503 7	30,666 2	59,669 5	57,069 -3	37,620 2	33,949 2	115,352 2

Inhibition of MLR by the cytotoxic treatment of responder cells from normal and chimaeric mice with anti-I-J and anti-Iat antibodies. An allogeneic MLR (allo-MLR) was set up with 2.5×10^5 spleen cells as responders and an equal number of MMC-treated SJA/9 spleen cells as stimulators in RPMI 1640 medium supplemented with 10% fetal calf serum and 5×10^{-5} M 2-mercaptoethanol in 96-well flat-bottomed microtitre plates. Cells were cultured for 5 days, and DNA synthesis assessed by pulsing with $1 \mu\text{Ci}$ of ^3H -thymidine for the final 16 h. The inhibitory activity of each antibody was studied by treating responder cells for 45 min at room temperature with a 1:10 dilution of ascites fluid from BALB/c *nu/nu* mice containing the monoclonal antibody followed by incubation with a 1:10 dilution of selected rabbit complement (C) for 30 min at 37°C . After extensive washing, cells were cultured with MMC-treated stimulator cells. The mean c.p.m. for five replicate cultures was determined, and the values shown are net c.p.m. (Δ c.p.m.), calculated by subtracting the sum of the c.p.m. for stimulator and responder cultures from the c.p.m. of a mixture of the two types of cells. Per cent MLR inhibition (% Inh.) was calculated as $[1 - (\Delta\text{c.p.m. for MLR with antibody} + \text{C} / \Delta\text{c.p.m. for MLR with C alone})] \times 100$. Note that the response of C3H and B6C3F₁, but not B6, was inhibited by treatment with two of the anti-I-J antibodies (1G8 and KN34) and with two of the anti-Iat antibodies (1L9 and 2L2). The MLR of the B6C3F₁ → C3H and C3H → B6C3F₁ chimaeras was inhibited by the same four antibodies that inhibited the parental C3H response, whereas no inhibition of the MLR was observed with spleen cells from the B6C3F₁ → B6 chimaera. The MLR of the B6 → B6C3F₁ chimaera and of the fully allogeneic B6 → C3H chimaera stimulated with SJA/9 was inhibited by only two of the antibodies (2L2 and KN34). Similar, even more prominent blocking of the MLR, with the same inhibitory pattern, was observed by the simple addition of $1 \mu\text{g ml}^{-1}$ of each antibody at the start of cultivation (data not shown).

β chains of T-cell receptors is unknown. Because the epitopes detected by these antibodies are selectively expressed on T cells, and as I region apparently controls the molecule, we designated these monoclonal reagents as anti-Iat antibodies. The present experiments used anti-I-J and anti-Iat antibodies, the properties of which have been described fully elsewhere⁸⁻¹¹.

Fully allogeneic and semi-allogeneic bone marrow chimaeras were produced according to the protocol of Singer *et al.*¹². The mice were maintained for over 3 months to establish a complete chimaeric state, which was confirmed at the time of each experiment by cytotoxicity assays using appropriate anti-H-2 reagents and by the unresponsiveness to both host and donor type stimulator cells in MLR. Sera from individual chimaeric mice were also typed to confirm the immunoglobulin allotype of donor origin.

The allo-MLR was performed by culturing 2.5×10^5 spleen cells from normal or chimaeric animals with an equal number of mitomycin C (MMC)-treated spleen cells of strain SJA/9 (*H-2^s*, *Igh-1^a*) in 0.2 ml for 5 days in 96-well microtitre plates. Although anyMHC-incompatible strains can be used as stimulators, SJA/9 was selected for use because both C3H and C57BL/6 (B6) responded very well to the SJA/9 cells, and the response of C3H was found to be inhibitable by a panel of anti-I-J and anti-Iat antibodies. Three anti-I-J (1G8, IgM; 4B11, IgM; and KN34, IgG2b) and four anti-Iat (1L9, IgM; 2L2, IgG2b; 14P, IgG1; and 74L, IgG2a) monoclonal antibodies were used in the present experiment to identify the epitopes on responding T cells. Before MLR cultivation, responder cells were treated for 45 min at room temperature with a 1:10 dilution of ascites fluid from BALB/c *nu/nu* mice containing each monoclonal antibody, then incubated at 37°C for 30 min with a 1:10 dilution of selected rabbit complement. For each antibody, less than 10% of responder cells were killed by the treatment. In the blocking experiments, the globulin fraction of ascites fluid was added to the MLR at a concentration of $1 \mu\text{g ml}^{-1}$ at the start of cultivation. Cells were pulsed with $1 \mu\text{Ci}$ of ^3H -thymidine (^3H -TdR) for the final 16 h of the 5-day cultivation.

Table 1 shows the inhibition of uptake of ^3H -TdR by C3H and (B6 × C3H)_{F₁} (B6C3F₁) responder cells stimulated with MMC-treated SJA/9 spleen cells by the treatment of the responder cells with two anti-I-J (1G8 and KN34) and two anti-Iat (1L9 and 2L2) antibodies. The response of B6 cells was not inhibitable by treatment with any of these antibodies. The MLR of spleen cells derived from the B6C3F₁ → C3H chimaera was also inhibitable by all four antibodies that were effective in inhibiting the response of parental C3H. None of the mono-

clonal antibodies was effective in inhibiting the response of spleen cells from the B6C3F₁ → B6 chimaera, even though the responding cells carried an *H-2^k* determinant, as detected by cytotoxicity testing with appropriate anti-class I antibodies. Thus, the T cells of F₁ stem-cell origin were found to express I-J^k and Iat^k epitopes only when they differentiated in the environment of an *H-2^k* but not an *H-2^b* recipient.

The experiments using parental T cells transferred into F₁ chimaeras gave more definitive results. Cells responding to SJA/9 derived from C3H → B6C3F₁ chimaeras were killed by all four antibodies (1G8, KN34, 1L9 and 2L2), while those from B6 → B6C3F₁ chimaeras expressed at least two of the epitopes detected by anti-I-J^k (KN34) and anti-Iat^k (2L2). The latter finding was especially interesting, as it indicates that the T cells of *H-2^b* stem-cell origin which differentiated in the *H-2^k* environment acquired at least some of the I-J^k and Iat^k phenotypes. The parental haplotype origin of the responding T cells was always confirmed by cytotoxicity testing with appropriate anti-H-2 reagents. In the fully allogeneic chimaera, the response of the B6 → C3H chimaera was inhibitable by the antibodies 2L2 and KN34, whereas the response of the C3H → B6 chimaera was not inhibitable by any of the antibodies. The same pattern of blocking of allo-MLR was observed by adding $1 \mu\text{g ml}^{-1}$ of these antibodies to the MLR culture (data not shown), which suggested the direct association of I-J and Iat epitopes with the allorecognition site of MLR responding T cells. The results indicate that I-J^k and Iat^k epitopes on MLR responder T cells are adaptively acquired in the chimaera, where allogeneic or semi-allogeneic stem cells differentiate into mature T cells under the selective influence of environmental MHC products.

Note, however, that the responses of F₁ → C3H and C3H → F₁ chimaeras were inhibited by the same four antibodies that were effective in inhibiting the MLR of normal C3H cells responding to the same SJA/9 stimulator, whereas the responses of B6 → F₁ and B6 → C3H chimaeras to the same stimulator were inhibited by only two of the four antibodies. This result suggests that the expression of I-J and Iat epitopes on the alloreactive T cells is determined essentially by two different factors: (1) the environmental MHC products in the host under which stem cells differentiate into T cells and acquire the alloreactive repertoire, probably in the thymus; (2) possibly the non-*H-2* genomic material originally possessed by the stem cell itself. Thus, B6 stem cells differentiated into T cells in a C3H environment are unable to express all the epitopes possessed by normal C3H responder cells.

We tested the above postulate by examining the pattern of inhibition of the MLR of bone marrow chimaeras transplanted

with *H-2*-compatible stem cells having different non-*H-2* background genes. We have shown previously that the inhibition pattern of both the syngeneic and allogeneic MLR by several anti-I-J antibodies differs among *H-2^k* strains, under the control of as yet unknown non-MHC genes⁸. Therefore, we constructed bone marrow chimaeras of B10.BR→C3H, in which *H-2* is identical, whereas non-*H-2* genes are different. Table 2 compares the inhibitory patterns produced by treatment with several anti-I-J and anti-Iat antibodies of B10.BR, C3H and B10.BR→C3H chimaeric T cells responding to the same SJA/9 stimulator. Whereas the C3H response was inhibited by 1G8, KN34, 1L9 and 2L2, the response of B10.BR was inhibited by only 1G8 and 74L, reflecting the difference in non-MHC genes between these two *H-2^k* strains. Exactly the same inhibitory patterns were observed in the blocking experiment in which antibodies were simply added to the MLR culture. The chimaeric T cells of B10.BR genotype differentiated in the C3H environment showed exactly the same pattern as B10.BR cells, but not the same as C3H, indicating that the expression of I-J and Iat epitopes is genetically determined in the case of *H-2*-compatible chimaeras.

These results are of importance in understanding why I-J products are detectable by serological and biochemical means^{5-7,11,13-16}, yet no genes have been identified at the prescribed position in the MHC^{3,4}. Several recent reports have discussed possible explanations for this discrepancy: (1) the I-J molecule is a modified α or β polypeptide of a class II MHC antigen, created by post-translational modification¹⁷; (2) I-J epitopes are created via an interaction between MHC and non-MHC genes, the latter being designated as *Jt* and mapped to chromosome 4²³; and (3) I-J is not the direct product of an MHC gene but is an inducible T-cell receptor for self *I*-region products^{11,18}.

Our findings indicate the following: (1) I-J and related Iat epitopes are present at the allorecognition site of MLR responder T cells; (2) I-J^k and Iat^k epitopes are inducible on chimaeric T cells derived from strains with a different MHC genotype but differentiated in the *H-2^k* environment; (3) the expression of I-J^k and Iat^k epitopes in chimaeric conditions is determined not only by the *H-2* phenotype of the host but also by the genomic material originally possessed by the stem cells; (4) the distribution patterns of I-J and Iat epitopes are determined by as yet unknown non-*H-2* genes. Based on these findings, we believe that I-J and Iat epitopes are not the mere markers of functional T-cell subsets, as originally proposed, but are associated with inducible T-cell receptors for alloantigen whose repertoire is determined by the environmental MHC products encountered during an early stage of T-cell differentiation. It has been proposed that such allorecognition is a version of self-MHC-restriction which is determined by selection in the thymus¹⁹⁻²².

Hayes *et al.*^{23,28} have suggested that the MHC and the *Jt* locus on chromosome 4 interact to give expression of the I-J epitope, probably through glycosylation. It is, however, difficult to explain the adaptive acquisition of polymorphic epitopes by means of simple glycosylation, because the F₁→B6 and C3H→B6 chimaeras cannot express I-J^k even though the stem cells should carry both *H-2* and *Jt* genes. It is puzzling that certain I-J epitopes are not detected on B10.BR responding T cells regardless of their presence on B6→C3H chimaeric T cells. Obviously there exists an as yet unknown regulatory mechanism whereby the fine organization of I-J epitopes at T-cell receptor sites is determined. Our results do not favour the postulate that I-J is a modified α or β chain of a class II antigen¹⁷, as it is unlikely that such pleiomorphic molecules can make structural changes by adapting to the environmental class II antigens.

It has been well documented that the functional MHC-restriction specificities of T cells are adaptable, changing according to the *H-2* environment in which they undergo early differentiation in the thymus^{21,22,24-26}. Yamauchi *et al.*²⁷ demonstrated adaptive changes in the functional MHC and immunoglobulin heavy-chain variable-region restriction specificities of sup-

Table 2 Inhibition of the allo-MLR of T cells from the B10.BR→C3H chimaera by anti-I-J and anti-Iat monoclonal antibodies in comparison with B10.BR and C3H T cells

Responder cells.		B10.BR		C3H		B10.BR → C3H	
Monoclonal antibody		Δc.p.m.	% Inh.	Δc.p.m.	% Inh.	Δc.p.m.	% Inh.
C alone		59,887	0	48,992	0	36,775	0
Anti-I-J 1G8		26,545	56	19,151	61	15,368	58
4B11		59,393	1	45,578	5	35,728	3
KN34		58,247	3	18,489	62	36,862	0
Anti-Iat 1L9		61,245	2	22,691	54	36,655	0
2L2		58,389	3	27,007	45	34,364	7
14P		57,059	5	54,272	6	34,328	7
74L		33,806	44	42,242	13	18,339	50

Inhibition of the MLR by treatment of responder cells from normal and *H-2*-compatible chimaeric mice with anti-I-J and anti-Iat antibodies. Allo-MLRs of B10.BR, C3H and B10.BR→C3H chimaeras stimulated by MMC-treated SJA/9 spleen cells were set up as described for Table 1. Note that the MLR of B10.BR is inhibited by two antibodies (1G8 and 74L), one of which (74L) does not inhibit the MLR of C3H. The inhibitory pattern of the B10.BR→C3H chimaera is exactly the same as that of B10.BR but differs from that of C3H.

pressor factors derived from chimaeric T cells, but were unable to detect the adaptive acquisition of allogeneic I-J determinants. Here, by using monoclonal antibodies that detect the polymorphic portion of the I-J molecule, we have provided evidence that serologically detectable I-J and Iat epitopes associated with the allorecognition structure are adaptively acquired in the chimaeric condition. We have shown previously that different anti-I-J monoclonal antibodies killed T cells responding to different alloantigens, which indicates the clonal distribution of these epitopes⁸. Thus, we conclude that I-J and Iat epitopes are not directly encoded by intra-*H-2* genes but are associated with the T cells' inducible receptors for the self class II antigens that determine the restriction specificity. This explains the apparent linkage of I-J and Iat phenotypes to class II alleles. Although *I-J* has yet to be mapped within the MHC, the present results provide new insight into the T-cell receptor for self antigens, and anti-Iat reagents should be useful in defining the restriction specificities and development of T cells.

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T cells with receptors for IgD

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The role of IgD in the immune response has been elusive, although its predominance on the cell surface suggests a receptor function. We have shown previously that euthymic but not athymic BALB/c mice, injected with IgD before antigen, exhibit enhanced antibody responses which can be transferred by T cells^{1,2}. Isotype-specific T cells have been reported to have both upward and downward immunoregulatory effects³⁻⁵. Here we demonstrate the existence of T cells with receptors for IgD, and show that exposure to IgD *in vivo* or *in vitro* significantly increases the number of T δ cells in the spleen and lymph nodes but not in the thymus. The kinetics of T δ -cell appearance *in vivo* parallels that of the immunoenhancing effect which occurs after injection of IgD. These T δ cells are of the Lyt 1⁺2⁻ T-cell phenotype.

Recently, we have found that mice bearing IgD-secreting plasmacytomas have enhanced humoral immune responses¹. We postulated that T cells bearing receptors for IgD (T δ) mediate the increased antibody production. The potential immunoregulatory activity of such a T-cell population is underlined by the fact that most peripheral B cells carry membrane IgD⁶.

We used sheep erythrocytes (SE) coupled to IgD in a rosetting assay to enumerate IgD-specific rosette-forming cells (IgD-RFC). The effects of overnight incubation at 37 °C of normal spleen cells with either a 1:10 dilution of TEPC 1017 ascites fluid (containing ~3 mg IgD ml⁻¹) or 0.04–20 µg of purified IgD ml⁻¹ are shown in Table 1 and Fig. 1. Fresh normal BALB/c spleen cells show 5 ± 0.8% IgD-RFC (*n* = 15) (result not shown), and after incubation with medium this percentage remains essentially unchanged. In contrast, exposure to IgD for

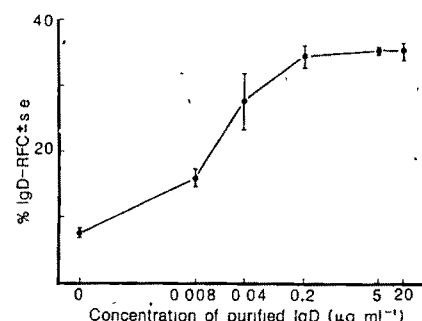


Fig. 1 Dose-response of IgD-induced IgD-RFC. Spleen cells from BALB/c mice were incubated at 37 °C for 18 h in 1 ml of MEM containing 2% FCS without or with purified IgD as described in Table 1. Following this incubation period, cells were washed twice in MEM and then examined in the IgD-RFC assay as described in Table 1.

18 h causes the percentage RFC to increase to >25%. Similarly purified splenic T cells incubated with IgD yield >25% RF (Table 1). The absence of an increase in percentage RFC on purification of T cells after panning on anti-immunoglobulin coated dishes could be the result of selective adherence of proportion of T δ cells on the membranes of IgD-expressing cells. Overnight incubation of splenic T cells with IgM- and IgG-containing ascites does not cause an increase in IgD-RFC. Addition of IgD to the RFC assay mixture significantly reduces the number of IgD-RFC detected (Table 1), whereas addition of IgM- and IgG-containing ascites has no detectable inhibitory effect.

Using a similar *in vitro* approach, we examined the effect of exposure to IgD on various cell populations (Table 2). Where spleen cells from normal BALB/c mice show a 5–6-fold increase in the number of IgD-RFC to 28% after *in vitro* incubation with IgD, spleen cells from mice injected with IgD *in vivo* show a mean value of 30% IgD-RFC with or without additional *in vitro* exposure to IgD. The failure of these cells to respond with further increase in the percentage of IgD-RFC suggests that the 30% value represents an upper limit to the inducible level of such cells.

Table 1 Specificity of IgD-RFC induced by exposure to IgD *in vitro*

Cell source	Addition during RFC assay*		Mean % IgD-RFC ± s.e. (n) after overnight incubation with:			
	IgD	IgM + IgG	Medium alone	IgD-containing ascites fluid	Purified IgD	IgM + IgG-containing ascites fluid
Spleen cells	—	—	8 ± 0.7 (9)†‡	27 ± 1.6 (10)†	29 ± 1.9 (19)‡	6 ± 1.3 (3)
Splenic T cells	—	—	9 ± 0.8 (4)§	31 ± 3.0 (4)§	27 ± 1.2 (8)	7 ± 0.9 (6)
Splenic T cells	+	—	—	6 ± 2.5 (3)	—	—
Splenic T cells	—	+	—	30 ± 0.05 (3)	—	—

Cells were obtained from BALB/c mice. Splenic T cells were purified by negative selection by panning spleen cells on Petri dishes coated with affinity-purified goat anti-mouse immunoglobulin¹⁴. The percentage of immunoglobulin-positive cells contaminating such purified T-cell suspension following this T-cell enrichment procedure was consistently <1%. Cells (2.5×10^6) were incubated at 37 °C for 18 h in 1 ml of minimal essential medium (MEM, Gibco) containing 2% fetal calf serum (FCS). Where indicated, the medium was supplemented with TEPC 1017 ascites fluid (3 mg IgD ml⁻¹, 0.04–20 µg ml⁻¹ purified IgD (pooled data from Fig. 1), or 10% IgM + IgG-containing ascites fluid obtained from mice bearing the MOPC 104E plasmacytoma. Following this incubation period, cells were washed twice and the cell concentration readjusted to 2.5×10^6 ml⁻¹ in MEM + 2% FCS. The cells were then used in the RFC assay. IgD was affinity-purified from ascites fluid taken from BALB/c mice bearing the IgD-secreting myeloma TEPC 1017, according to methods described previously¹, then it was coupled to SE using the CrCl₃ coupling method¹. Coupling of IgD to SE was confirmed by both positive haemagglutination and indirect immunofluorescence assays using a rabbit anti-mouse Ig antiserum. IgD-RFC were enumerated using a modified method of Chen *et al.*¹⁶. Briefly, 0.2 ml of 1% IgD-SE was mixed with 0.1 ml of assay cells (2.5×10^5) at 37 °C for 15 min. Cells were then centrifuged at 500 r.p.m. (200g) for 5 min and incubated further at 4 °C for 45 min or overnight. Immediately before scoring RFC, the lymphocytes were stained using 0.025 ml of a 1% toluidine blue solution. Lymphocytes surrounded by over three indicator cells were scored as rosettes and the result expressed as % RFC. In each experiment, the percentage of cells forming rosettes with SE was determined and always found to be <2%.

* Where indicated, IgD-containing, or IgM plus IgG-containing ascites fluid was added to samples to a final concentration of 6%.

† For medium alone compared with IgD-containing ascites fluid, *P* < 0.0001.

‡ *P* < 0.0001; § *P* = 0.001; || *P* < 0.001, ¶ *P* = 0.001.

Table 2 *In vitro* induction of T δ cells following overnight incubation with IgD-containing ascites fluid

Cell source	<i>In vivo</i> pretreatment	Medium alone	Mean % IgD-RFC $\pm$ s.e. following overnight incubation with IgD-containing ascites fluid	P
Spleen cells	—	8 $\pm$ 1.2	28 $\pm$ 1.9	<0.0001
Spleen cells	TNP-KLH*	11 $\pm$ 1.7	30 $\pm$ 2.4	0.001
Spleen cells	IgD†	30 $\pm$ 1.7	31 $\pm$ 2.4	NS
Splenic T cells	—	8 $\pm$ 0.7	30 $\pm$ 2.9	0.01
Splenic Lyt 1 ⁺ 2 ⁺ T cells	—	—	40 $\pm$ 2.1	<0.0001‡
Splenic L3T4 ⁺ T cells	—	—	8 $\pm$ 1.9	NS‡
Splenic B cells	—	5 $\pm$ 0.7	8 $\pm$ 1.4	NS
Lymph node cells	—	5 $\pm$ 0.9	42 $\pm$ 3.9	0.006
Thymocytes	—	5 $\pm$ 0.3	8 $\pm$ 1.5	NS
C B20 spleen cells	—	8 $\pm$ 0.5	31 $\pm$ 2.2	<0.0001

Cells were incubated in MEM+2% FCS with or without IgD-containing ascites fluid as described in Table 1. The results are the means for 3–6 assays. Except for C B20 spleen cells, the cells were obtained from BALB/c mice. Splenic T cells were purified as described in Table 1. Using purified T-cell suspensions as the starting population, Lyt 1⁺2⁺ and L3T4⁺ T-cell subpopulations were isolated by complement-mediated cytotoxicity using the monoclonal antibodies anti-Lyt 2.2 (19/178), provided by Dr U Hammerling (Sloan-Kettering Institute, New York), and anti-L3T4 (GK1.5)¹⁷, obtained from Dr F. Fitch (University of Chicago, Illinois) respectively. Splenic B cells were purified by complement-mediated cytotoxicity of T cells using a cocktail containing anti-Thy-1.2 (6.80), provided by Dr U Hammerling, anti-Lyt 1.2 (C3PO) provided by Dr J. Klein (Max Planck Institute, Tübingen) and anti-L3T4 (GK1.5).

* Mice were injected intravenously (i.v.) with 100 μ g TNP-KLH 12 and 5 days before the day of assay.

† Mice were injected i.p. with 0.5 ml of IgD-containing ascites fluid 7 and 1 day before the day of assay.

‡ P values determined by comparing the mean % IgD-RFC following overnight incubation with IgD with the mean % RFC for splenic T cells following overnight incubation in medium alone

The T-cell lineage of the IgD-RFC was investigated further by examining the ability of specific T-cell subsets to form rosettes with IgD-SE. As shown in Table 2, isolated Lyt 1⁺2⁺ but not L3T4⁺ T cells yield significantly increased numbers of IgD-RFC after overnight incubation with IgD. These results suggest that most, if not all, the IgD-RFC are Lyt 1⁺2⁺, L3T4⁺ T cells—that is, they have the helper cell phenotype. However, the possibility that some RFC may be Lyt 2⁺ cannot be excluded; further studies are needed to determine whether the incidence of IgD-RFC in Lyt 2⁺ cells can be increased if they are exposed to IgD in the presence of other cell types. Marcelletti and Katz⁷ have recently reported that optimal induction of IgE-binding, Lyt 2⁺ T δ cells is dependent on the presence of B cells. The majority of T α and T β cells induced by exposure to IgA and IgE respectively, are Lyt 2⁺ (refs 8, 9).

The percentage of IgD-RFC in lymph-node cell suspensions also increases (from 5 to 42%) after incubation with IgD. In contrast to peripheral T cells, however, thymocytes show only background levels (5–8%) of IgD-RFC before and after *in vitro* exposure to IgD. Similarly, when isolated splenic B cells are incubated overnight with IgD, no significant increase above background numbers of IgD-RFC is seen.

The induction of IgD-RFC is not allotype-specific as spleen cells from C.B20 mice, which express the *b* allotype of IgD, are as responsive as BALB/c spleen cells to TEPC 1017-derived IgD of allotype *a*. In addition, C.B20 mice bearing the TEPC 1017 plasmacytoma show a significant enhancement of the 6-day primary response to trinitrophenyl-keyhole limpet haemocyanin (TNP-KLH) (523 $\pm$ 134% of the control for 19S plaque-forming cells (PFC) and 341 $\pm$ 141% of control for 7S PFC).

Although these data established that exposure to IgD *in vivo* and *in vitro* causes the appearance of IgD-RFC, the phenotype of the *in vivo*-induced RFC remained to be determined. Table

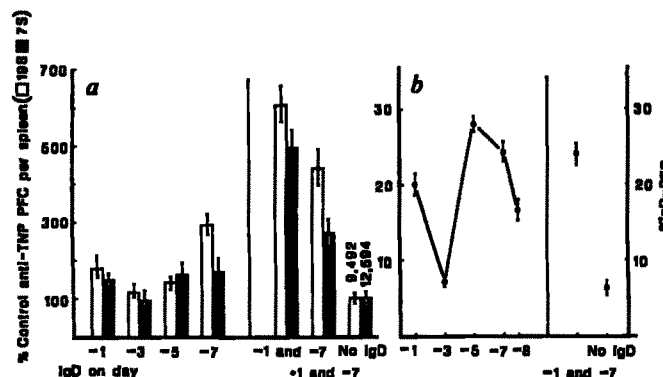


Fig. 2 *a*, Kinetics of IgD-induced enhancement of antibody response to TNP-KLH. Results are presented as % of control responses ($\pm$ s.e.). The geometric means of responses in control mice for 19S and 7S PFC per spleen (100%) are shown above the bars. BALB/c mice were injected with 0.5 ml of IgD-containing ascites fluid on the days indicated ($n=3-11$). Mice were primed on day 0 with 100 μ g of TNP-KLH and their anti-TNP PFC responses were measured 5 days later. Anti-TNP splenic PFC were assayed by the slide modification of the technique of Jerne *et al.*¹⁸. TNP-SE were prepared using the method described by Rittenberg and Pratt¹⁹. IgG-producing cells (that is, 7S responses) were developed with rabbit anti-mouse immunoglobulin in the complement and goat anti- μ in the agar. *b*, Kinetics of appearance of T δ cells after i.p. injection of IgD-containing ascites fluid. BALB/c mice were injected with 0.5 ml of IgD-containing ascites fluid on the days indicated. Spleen cells were obtained on day 0 and the percentage of IgD-RFC determined ($n=3-6$).

3 shows that ~30% IgD-RFC are obtained in both unfractionated and Lyt 1⁺2⁺ splenic T cells following injections of IgD. The induction of IgD-RFC *in vivo* also parallels the *in vitro* results in that >30% RFC are found in lymph nodes and only 3% RFC in thymus. The results in Tables 2 and 3 suggest strongly that injection of antigen also causes a significant increase in the number of splenic IgD-RFC. Further characterization of these RFC is underway.

The kinetics of appearance of T δ cells after intraperitoneal (i.p.) injection of IgD was studied in parallel with the humoral immune responsiveness of similarly treated mice (Fig. 2). The responses of animals injected with IgD on day -1, -5 or -7 or on both days +1 or -7 were higher than those of untreated mice. In contrast, the responses of mice injected with IgD 3 days before injection of antigen were not augmented. The presence or absence of augmentation of the PFC response is in complete agreement with the presence or absence of increased numbers of T δ cells in the spleen. However, the magnitude and kinetics of the two phenomena differ somewhat. The percentage of T δ cells rises to a near maximum within 1 day after IgD injection, falls to background levels by day 3 and then rises again to maximal levels by day 5. The percentage of T δ cells found in the spleens 1 day after a second injection of IgD is as high as after the first injection. However, the magnitude of the PFC response induced after two injections of IgD is much greater than at any time after a single injection. These findings suggest strongly that T δ cells are at least as readily induced as T cells bearing Fc receptors for other immunoglobulin isotypes^{8,10}, and that T δ cells have an augmenting effect on the 19S and 7S antibody responses. The observation that maximal augmentation of immune responses *in vivo* requires two injections, whereas maximal numbers of T δ cells are observed after a single injection of IgD, may reflect a ceiling effect with respect to the percentage of splenic T cells capable of expressing receptors for IgD. It is also possible that individual T δ cells either express more receptors for IgD or become more responsive to target B cells after repeated exposure to IgD.

Table 3 Phenotype and tissue distribution of T δ cells induced *in vivo*

Cell source	<i>In vivo</i> pretreatment with IgD- containing ascites fluid	Mean % IgD-RFC $\pm$ s.e. (n)	P
Spleen cells	+	23 $\pm$ 1.4 (8)	<0.0001
	—	5 $\pm$ 0.8 (6)	
Splenic T cells	+	30 $\pm$ 4.7 (5)	0.02
	—	4 $\pm$ 0.4 (4)	
Lyt 1 ⁺ 2 ⁺ splenic T cells	+	27 $\pm$ 1.3 (5)	<0.0005
	—	5 $\pm$ 0.3 (3)	
Lymph node cells	+	32 $\pm$ 2.5 (5)	<0.005
	—	12 $\pm$ 1.3 (4)	
Thymocytes	+	3 $\pm$ 0.8 (4)	NS
	—	5 $\pm$ 0.5 (4)	
TNP-KLH-primed spleen cells	—	12 $\pm$ 1.2 (7)	<0.005
Unprimed spleen cells	—	5 $\pm$ 0.6 (7)	

Cells were obtained from BALB/c mice. Splenic T cells and Lyt 1⁺2⁺ splenic T cells were purified as described in Tables 1 and 2. Where indicated, spleen cells were obtained from BALB/c mice immunized with 100 μ g TNP-KLH 5 days before the day of assay. Where indicated, mice were injected twice (i.p.) with 0.5 ml of IgD-containing ascites fluid 7 and 1 days before the day of assay.

Results from other studies show that augmentation of the humoral immune response in normal mice can be achieved by transfer of unfractionated spleen cells, unfractionated T cells² or purified Lyt 1⁺2⁺ T cells from donors preinjected with IgD¹¹. The mechanism of action of these IgD-induced T-helper cells has not yet been established, but, in the absence of polyclonal B-cell activation¹, it is expected that interaction of antigen with IgD on the surface of the B cell activates T δ cells, which then exert their augmenting effect. Whether, as suggested previously¹, this effect is mediated through down-regulation of IgD synthesis, a known consequence of B-cell activation by antigen or mitogen^{12,13}, or through the release of a soluble mediator, remains to be established. It is of interest to speculate that the small but significant increase in T δ cell number which occurs after injection of antigen is the result of stimulation by antigen-IgD complexes. Our demonstration of IgD-rosetting T cells provides evidence for the existence of a mechanism in which T-cell help is given to all IgD-positive B cells in an antigen-dependent though not antigen-specific fashion.

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Platelet secretory products inhibit lipoprotein metabolism in macrophages

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Macrophages possess a receptor that binds low-density lipoproteins (LDL) containing lysine residues modified by acetylation (Ac LDL), acetoacetylation (AcAc LDL) or malondialdehyde treatment^{1–3}. This receptor (referred to as the Ac LDL receptor or scavenger receptor) internalizes the bound lipoprotein. As a consequence, massive amounts of cholesteryl esters accumulate so that macrophages in culture resemble foam cells found in atherosclerotic lesions^{4,5}. In an effort to identify an unmodified mammalian macromolecule that binds to the Ac LDL receptor, we investigated whether platelet secretory products affect the receptor-mediated endocytosis of chemically modified lipoproteins. Platelets are a potential source of such activity because they exist in close association with foam cells in developing atherosclerotic lesions^{6,7}. Our study demonstrates that human blood platelets secrete a product that inhibits the binding and uptake of AcAc LDL by mouse peritoneal macrophages and the subsequent accumulation of cholesteryl esters. This is the first indication that an endogenous macromolecule interacts with Ac LDL receptor on macrophages.

Platelet secretory products, obtained from platelets of freshly drawn human blood (see Fig. 1 legend), inhibited the cellular uptake of ¹²⁵I-AcAc LDL (Fig. 1a) and the subsequent degradation of the iodinated lipoprotein to trichloroacetic acid-soluble material (Fig. 1b) by mouse peritoneal macrophages. The secretory products also inhibited the uptake and degradation of ¹²⁵I-Ac LDL (data not shown). Preincubation of the macrophages with 100 μ g ml⁻¹ platelet secretory protein for 16 h followed by washing did not affect the subsequent cellular uptake and degradation of ¹²⁵I-AcAc LDL, indicating that the platelet products did not inhibit the uptake by down-regulating the Ac LDL receptor. Previous studies have shown that cultured macrophages bind AcAc LDL at 4 °C, a temperature at which internalization and subsequent proteolytic degradation do not occur⁵. We found that at 4 °C, the secretory products from platelets inhibited ¹²⁵I-AcAc LDL binding to macrophages; 59 μ g ml⁻¹ secretory products were needed for 50% inhibition (data not shown). The platelet-derived inhibitor demonstrated specificity for interaction with the Ac LDL receptor in that it had little effect on the uptake and degradation of ¹²⁵I-labelled β -migrating very-low-density lipoproteins (β -VLDL) (Fig. 1a, b, respectively) by the β -VLDL receptor on mouse peritoneal macrophages⁵. Similarly, the platelet secretory products, at concentrations shown to inhibit the uptake and degradation of ¹²⁵I-AcAc LDL by macrophages, had little effect on the uptake of ¹²⁵I-LDL by cultured human fibroblasts (data not shown). Thus, platelet secretory products act to inhibit selectively the binding, uptake and degradation of ¹²⁵I-AcAc LDL by macrophages.

The cholesterol liberated following receptor-mediated uptake of AcAc LDL by macrophages is re-esterified in the cytoplasm⁵. Cholesteryl ester synthesis can be readily assayed by including ¹⁴C-oleate in the culture medium and measuring the formation of radiolabelled cholesteryl oleate¹. We found that the platelet secretory products inhibited the AcAc LDL-induced formation of cholesteryl ¹⁴C-oleate by mouse peritoneal macrophages (Fig. 2). Therefore, one functional consequence of the platelet-derived inhibitory activity is the prevention of AcAc LDL-mediated cholesteryl ester formation and accumulation.

Agents and conditions known to affect the release of platelet secretory products were examined to determine their ability to release the inhibitor of AcAc LDL degradation (Fig. 3). Whereas the thrombin-induced secretory products inhibited the degradation of ¹²⁵I-AcAc LDL by mouse peritoneal macrophages, little

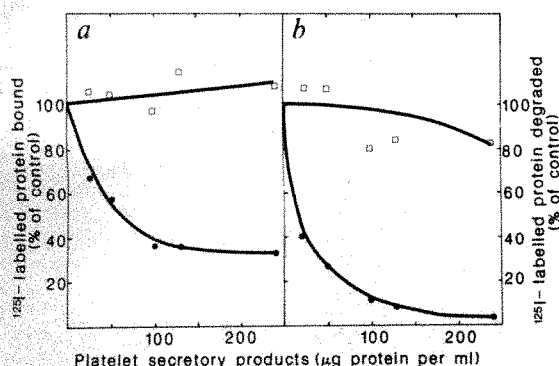


Fig. 1 Effect of platelet secretory products on the uptake (a) and degradation (b) of ^{125}I -AcAc LDL (●) or ^{125}I - β -VLDL (□) by mouse peritoneal macrophages.

Methods. Secretory products were obtained from the platelets of freshly drawn human blood. Platelets were collected and washed as described previously¹⁴ and, except where noted, suspended to a concentration of $3\text{--}5 \times 10^9$ platelets per ml in a solution containing 1 mM EDTA, 10 mM HEPES and 0.15 M NaCl pH 7.4 (EHS buffer). The washed platelets were induced to secrete by treatment with 0.5 U ml^{-1} human α -thrombin (a gift from Dr John Fenton III) for 5 min at ambient temperature, after which time the thrombin was inhibited by the addition of hirudin at 1 U ml^{-1} . The platelets were then removed by centrifugation at $10,000g$ for 15 min at 4°C and discarded. The supernatant, containing the secretory products, was dialysed against EHS buffer, concentrated by filtration (YM-10 filter, Amicon Corporation) and frozen until needed. Mouse peritoneal macrophages were collected from unstimulated mice and dispensed into 16-mm Petri dishes (5×10^5 cells per dish) as described previously¹⁵. The dishes were incubated for 2 h at 37°C and washed three times with Dulbecco's modified Eagle's medium (DMEM) to remove non-adherent cells. The cultured macrophages were incubated overnight in DMEM containing 20% fetal calf serum and washed once with DMEM before use. To measure the uptake and degradation of the labelled protein, each 16-mm dish of macrophages received 0.3 ml DMEM containing 10% human lipoprotein-deficient serum, the indicated concentrations of platelet secretory products and either $1 \mu\text{g ml}^{-1}$ ^{125}I - β -VLDL or $1 \mu\text{g ml}^{-1}$ ^{125}I -AcAc LDL. The dishes were then incubated for 5 h at 37°C . The amount of ^{125}I -labelled protein taken up and degraded by the cells was determined as described previously¹⁵. Procedures for the isolation and iodination of the AcAc LDL and β -VLDL and the modification of the LDL by acetoacetylation have also been described elsewhere¹⁵.

inhibitory activity was detected when platelets were incubated without thrombin, when thrombin was mixed with hirudin before addition to platelets, or when platelets were preincubated with prostacyclin. This demonstrates that the proteolytic activity of thrombin and the functional activity of platelets are required for the expression of the inhibitory activity. Two additional platelet agonists, the Ca^{2+} ionophore A23187 and collagen, were found to release an amount of inhibitor equivalent to that released by thrombin. These findings suggest that the inhibitor is secreted from the platelet storage organelles⁸, probably α -granules, which are the primary source of protein secreted in response to the three agonists tested⁹. Because the concentration of secretory products used for the experiment in Fig. 3 was equivalent to that induced from platelets at 2.5×10^8 per ml, we conclude that the inhibitor is secreted in sufficient concentrations at physiological platelet counts ($3\text{--}4 \times 10^8$ platelets per ml) to affect the binding activity of the Ac LDL receptor on macrophages.

Initial attempts to characterize the inhibitor of AcAc LDL uptake and degradation showed that the activity from thrombin-activated platelets persisted after samples were frozen and thawed twice. This activity was not affected by incubating the platelets for 30 min at temperatures between 4°C and 70°C and did not pass through filters (XM 100, Amicon Corporation) with an exclusion limit of relative molecular mass 100,000 (data not shown). From these observations, we tentatively conclude that the inhibitor is a stable secretory product of platelets and is

Fig. 2 Inhibition of AcAc LDL-mediated cholesterol esterification in mouse peritoneal macrophages by platelet secretory products. Each 16-mm Petri dish received 0.3 ml of DMEM containing 0.2 mM ^{14}C -oleate (oleate albumin complex), $4 \mu\text{g}$ protein per ml of AcAc LDL, and the indicated concentration of platelet secretory products. After incubation for 16 h at 37°C , the cellular content of ^{14}C -oleate was determined by TLC¹⁵.

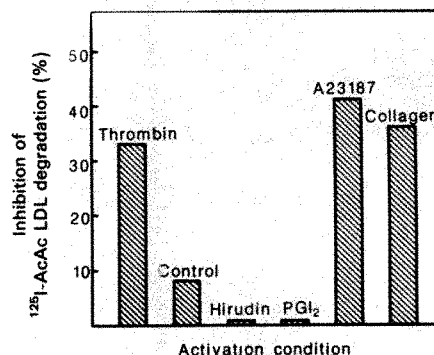
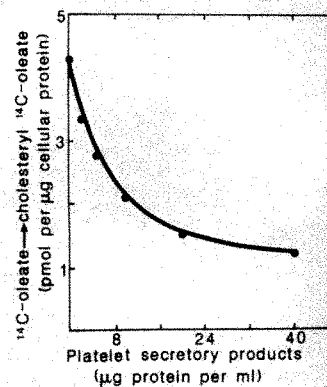


Fig. 3 Platelet secretion is required for the expression of the inhibitor of ^{125}I -AcAc LDL degradation by macrophages. Platelets from normal volunteers were isolated and suspended in EHS buffer (10^9 platelets per ml). Aliquots of 1.5 ml were treated as indicated for 5 min at ambient temperature and centrifuged at $1,000g$ for 15 min to sediment the platelets. In some cases, hirudin was added immediately after the 5-min incubation. The supernatant was removed, centrifuged at $15,000g$ for 2 min and assayed for inhibition of ^{125}I -AcAc LDL degradation as described in Fig. 1 legend, using a 1:4 dilution of the platelet supernatant. Conditions: thrombin, 0.5 U ml^{-1} ; control, no addition; thrombin plus hirudin, thrombin (0.5 U ml^{-1}) preincubated with hirudin (1 U ml^{-1}) before addition to platelets; PGI_2 , platelets preincubated with 50 nM prostacyclin for 10 min at ambient temperature before addition of thrombin; A23187, $1 \mu\text{M}$ A23187; collagen, $20 \mu\text{g ml}^{-1}$ collagen.

associated with a macromolecule(s) of high relative molecular mass.

Although the physiological function of a platelet-derived inhibitor of the Ac LDL receptor on macrophages is unknown, we suggest two possibilities. One is that this inhibitor regulates accumulation of cholesterol in these cells. In atherosclerotic lesions, cholesteryl esters accumulate in macrophage-derived foam cells^{5-7,10}. Although the mechanism of lipid uptake is unknown, it has been suggested that LDL can be modified *in vivo* so that they can be recognized by the Ac LDL receptor^{3,5,11,12}. If such modifications cause cholesterol accumulation in foam cells, the platelet secretory products released at the site of an atherosclerotic lesion could serve to dampen the rate of cholesterol accumulation. An alternative possibility is that the platelet-derived inhibitor could activate macrophages. Binding of ligands to the Ac LDL receptor on macrophages induces the secretion of several proteins, including the Ca^{2+} -dependent neutral protease, a plasminogen activator and a cytolytic factor¹³. The inhibitor from platelets may induce similar secretions.

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Cultured human endothelial cells express platelet-derived growth factor B chain: cDNA cloning and structural analysis

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Vascular endothelial cells have a central role in various pathophysiological responses such as acute inflammation, wound healing and atherogenesis. The anatomical position of endothelial cells between blood leukocytes and the surrounding vascular smooth muscle cells or stromal fibroblasts may intensify and focus the effects of released endothelial cell products. Endothelial cells in culture produce a platelet-derived growth factor (PDGF)-like mitogen¹. PDGF purified from platelets is a basic protein with an apparent relative molecular mass (M_r) of ~30,000 (reviewed in refs 2, 3) and is believed to comprise two polypeptide chains, PDGF-A and PDGF-B⁴ (also referred to as PDGF-1 and PDGF-2; refs 5, 6). Sequence analysis of PDGF B chain has revealed a striking homology with the predicted sequence of p28^{sis}, the transforming protein of simian sarcoma virus^{4,6,7}. *sis*-Homologous transcripts have been detected by Northern blot analysis of RNA from cultured endothelial cells⁸. However, there are no structural data available on either the protein product or the messenger RNA to establish the identity of the endothelial-derived mitogen with either chain of PDGF. Here we report the isolation and complete sequence analysis of a *sis*-homologous complementary DNA clone from human endothelial cells, providing an opportunity to study the structure of *sis* as transcribed by a normal (untransformed) cell. Our results establish that normal human endothelial cells in culture express the B chain of PDGF, and that endothelial-derived PDGF B chain is synthesized as a predicted precursor polypeptide of M_r 27,281.

A prominent *v-sis* hybridizing band of about 3.5 kilobases (kb) was detected in Northern blots of cytoplasmic RNA from cultured human umbilical vein endothelial (HUVE) cells⁹, a simian virus 40 (SV40)-transformed endothelial cell line¹⁰ and a bovine aortic⁹ endothelial cell strain (Fig. 1A). These bands are of a similar size to that of an RNA species isolated from a human osteosarcoma cell line (HOS), previously reported to be ~4.2 kb¹¹. A diffuse minor band of 2.7 kb was also observed. No *v-sis* hybridizing bands were detected in RNA preparations from cultured human dermal fibroblasts or from a B-lymphoblastoid cell line.

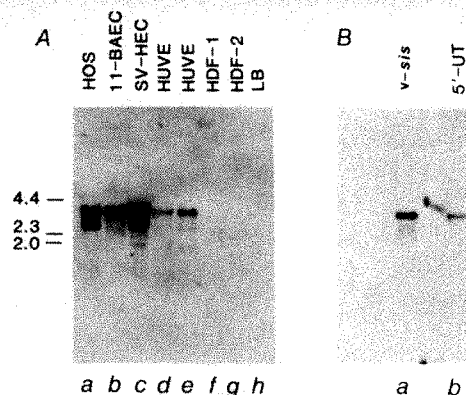
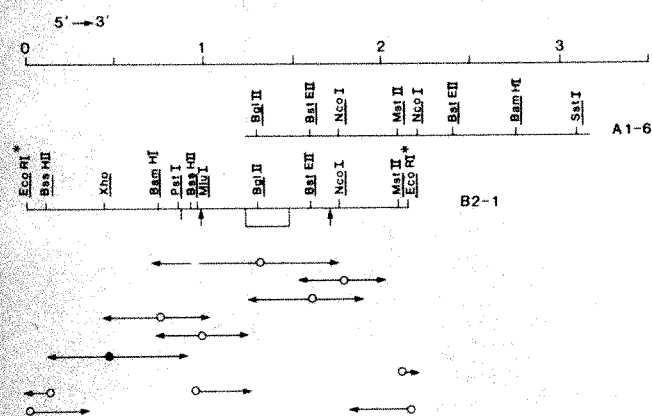


Fig. 1 A, Expression of *c-sis* in cultured vascular endothelial cells. *a-c*, 50 μ g each of cytoplasmic RNA from a human osteosarcoma cell line (HOS), cultured bovine aortic endothelial cells (BAEC) and an SV40-transformed endothelial cell line, respectively; *d, e*, 10 and 20 μ g, respectively, of cytoplasmic RNA from human umbilical vein endothelial cells; *f, g*, 50 μ g of cytoplasmic RNA from each of two strains of human dermal fibroblasts; *h*, 5 μ g of poly(A)⁺ RNA from the lymphoblastoid line LB. The molecular sizes (kb) of ³²P 3'-end-labelled *Hind*III-digested phage λ DNA, processed in parallel with the RNA samples, are indicated on the left. B, Hybridization probes from the 5'-untranslated region of the B2-1 clone and the B-chain coding region identify the same transcript in RNA from HUVE cells. In each lane, 5 μ g of poly(A)⁺ RNA from HUVE cells was analysed by RNA blotting techniques³⁰. Following transfer to nitrocellulose, the lanes were separated and independently analysed with: *a*, a coding-region probe (*v-sis*) or *b*, a probe from the 5'-untranslated (5'-UT) region (an *Eco*RI-*Hin*FI restriction fragment, nucleotides 1-280).

Methods. Total cytoplasmic or poly(A)⁺ RNA was prepared as described elsewhere³⁰. The RNAs were denatured with formaldehyde and electrophoresed in a 1% agarose gel, transferred to nitrocellulose, and probed with nick-translated cDNA inserts as described elsewhere³⁰. The filter was washed in 0.5 $\times$ SSC at 68 $^{\circ}$ C and exposed to film as described previously³¹.

To determine the nucleotide sequence of the *sis*-homologous 3.5-kb transcript, a cDNA corresponding to the endothelial cell mRNA was cloned and its primary structure determined. Restriction mapping and Southern blot hybridization with the *v-sis* probe were used to establish the overlapping orientation of two cDNA clones from the random hexanucleotide (B2-1) and the oligo(dT)-primed (A1-6) libraries (Fig. 2). The 5' clone (B2-1) contains the *v-sis*-homologous region and extends 860 base pairs (bp) farther 5' than a previously described cDNA clone derived from the HUT 102 neoplastic T-cell line¹². The 3' clone A1-6 ends just 3' to an *Sst*I site located ~500 bp upstream from the polyadenylation site (based on a restriction map comparison with the HUT 102 cDNA). Together these clones span 3.2 kb and by inference account for most of the 3.5-kb *sis*-homologous transcript observed on Northern blots of HUVE cytoplasmic RNA.

Sequence analysis of the B2-1 clone confirms the presence of a region of *v-sis* homology containing an open reading frame that encodes the B chain of PDGF; it also reveals an exceptionally long 5'-untranslated region (Fig. 3A). The *v-sis*-homologous region begins at position 1,053 and continues to the 3' end of this clone. Over the region encoding the B-chain protein sequence, the HUVE cDNA clone is identical in nucleotide sequence both to the previously sequenced clone from the HUT 102 T lymphoma cell line¹² and to the exons of the normal human *c-sis* gene^{4,13,14}. A potential initiation codon, ATG (position 989), is in-phase with the B-chain open reading frame and the coding region would extend 723 bp before terminating at position 1,712. The predicted primary translation product for the B chain is 241 amino acids long (M_r 27,281). The sequence in Fig. 2 must encode the entire precursor for the B chain because, as in HUT 102, an in-frame translational stop codon is located 117 bp upstream (position 872) from the open reading frame.



Located at the N-terminus of the predicted precursor is a hydrophobic sequence (bases 998-1,036) encoding residues 4-16, which may serve as a signal for secretion. The protein encoded by *v-sis* contains a similar signal sequence derived from the retroviral envelope gene. Construction of deletion mutants in the putative *v-sis* signal region was correlated with the loss of transforming ability¹⁵. The B chain of PDGF, as isolated from platelets, is known to be both cysteine-rich and highly basic^{2,3}. The predicted precursor is interesting in that the putative amino-terminal 'pro' region has no cysteines and is markedly acidic. In contrast, the extreme carboxy terminus, which also appears to be removed proteolytically from mature B chain⁴, contains cysteine residues and is highly basic.

The B2-1 HUVE clone extends 293 bp upstream from the reported 5' end of the genomic sequence¹⁶. To confirm that the 5' HUVE cDNA region is actually part of the *c-sis* gene, a probe encoding nucleotides 1-280 was hybridized to HUVE poly(A)⁺ RNA and compared with a probe defining the coding region

Fig. 3 A, Nucleotide sequence of the B2-1 cDNA clone of the PDGF B chain. The nucleotide sequence is shown, with the predicted amino acids (standard three-letter code) below the respective codons of the B-chain open reading frame. Mature PDGF B chain, isolated from platelets, appears to extend from an N-terminal serine (nucleotides 1,232-1,235) to a C-terminal threonine (nucleotides 1,556-1,558), indicated by single asterisks. The three ATG codons located 5' to the B-chain open reading frame are underlined, as is the TGA codon in-frame with the translational start site for the B chain. The 5' end of the presently reported genomic sequence¹⁶ is indicated by the solid arrowhead; the 5' end of the previously reported HUT 102 cDNA sequence¹² is coincident with the underlined TGA codon. Nucleotides 1,743 to 1,891 (indicated by Δ) in the 3'-untranslated region of the endothelial cDNA clone are not found in *v-sis*, but are also present in the HUT 102 cDNA¹², suggesting that alternative splicing may occur in the 3'-untranslated region, or that a deletion event occurred in the woolly monkey genome (the presumed source of *v-sis*). **B, Distribution of translation termination codons in human endothelial cells.** The positive codons are indicated (vertical lines) orientations. The four 5'-proximal of the text are indicated by boxes; the fourth most 5'-proximal ATG codon

[illegible]

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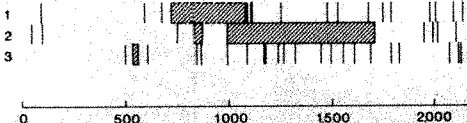


Fig. 2 (Left) Restriction map and sequencing strategy. Restriction maps of overlapping A1-6 (oligo(dT) library) and B2-1 (random library) cDNA clones were determined by Southern blotting with a *v-sis* probe and restriction analysis. The location of the B-chain precursor is indicated by arrows on the B2-1 clone; the region of the cDNA corresponding to the protein sequence⁴⁻⁷ of PDGF is indicated by an open box. The sequencing strategy is outlined below the B2-1 restriction map. Arrows indicate the direction of sequencing using the Maxam-Gilbert³² chemical degradation method. Asterisks indicate that the *EcoRI* sites on the B2-1 clone have been added during the cloning process.

Methods. HUVE cells were cultured as described³³. Construction of the cDNA libraries is presented in detail elsewhere³⁴. Briefly, cells were collected and total RNA prepared by lysis in buffer containing 6 M guanidine-HCl. Poly(A)⁺ mRNA was isolated from total endothelial cell RNA by oligo(dT)-cellulose column chromatography³⁰; cDNA was synthesized by the method of Okayama and Berg³⁵ as modified by Gubler and Hoffman³⁶, using oligo(dT)₁₂₋₁₈ as primer for first strand synthesis (oligo(dT) library). A second cDNA pool was synthesized using a random mixture of hexanucleotides as primer for the first strand synthesis (random library). Recombinant λ gt11 phage clones³⁸ containing inserts homologous to c-sis were isolated from the cDNA libraries by screening with the 950-bp *Xba*I-*Pst*II fragment of the retroviral transforming gene v-sis²⁵. The probe was radiolabelled using an oligonucleotide primer labelling method³⁷.

(*v-sis*) by Northern blotting. The two probes hybridize to an identical 3.5-kb transcript (Fig. 1B). Furthermore, both probes also hybridize to the same 24-kb *Eco*RI fragment in a genomic Southern blot analysis (not shown). In the region of the overlap, the only major differences between the published 5' genomic sequence¹⁶ and the HEC cDNA sequence are the eight nucleotides at the 5' end of the genomic clone (cDNA position 292-299). These nucleotides were probably added during construction of the genomic library¹⁷ and the 5' continuation of the cDNA is transcribed, at least in part, from the continuation of the present 5' exon.

The 5'-untranslated region of the PDGF B-chain transcript is unusually long (at least 983 bp). Most 5'-untranslated regions are less than ~150 bp long¹⁸, although there are notable exceptions. Specifically, *c-myc*, another cellular homologue of a retroviral transforming gene (whose expression is activated by PDGF¹⁹), has a 550-bp 5'-untranslated region²⁰. Another mitogen, murine preproepidermal growth factor, also has a long (353 bp) 5'-untranslated region²¹, and genomic sequence analysis suggests that the insulin-like growth factor II precursor has ~1 kb of 5'-untranslated region²². The transcript is also unusual in that translation begins at the fourth most 5'-proximal ATG codon (position 989, Fig. 3B). Recently, a consensus sequence (CC(A or G)CCATGG) for translation initiation has been defined¹⁸, in which purine nucleotides three positions upstream and one position downstream from the ATG are particularly important²³. Only the ATG in the B-chain reading frame meets both these criteria (TCGGCATGA). The second open reading frame could encode a protein of *M_r* 12,529. A search of the GenBank sequence database revealed no significantly (non-*sis*-related) homologous sequences. In addition, analysis of codon usage²⁴ suggests that this open reading frame is unlikely to encode a translated protein.

The extreme length of the 5'-untranslated region and the presence of three other ATG codons may be important in the physiological regulation of PDGF expression. Interestingly, both the naturally transforming retrovirus^{15,25} and the experimentally transforming HUT 102 cDNA²⁶ clone delete the other upstream ATG codons. Similarly, when *c-sis* genomic constructions lacking the complete 5'-untranslated region are placed under the transcriptional control of a retroviral long terminal repeat, the constructs acquire high-titre transforming activity¹⁶.

PDGF as purified from human platelets contains a second polypeptide that is less homologous to *v-sis* (A chain)⁴, and it is thought that human PDGF is predominantly a 1:1 disulphide-linked heterodimer of A and B chains^{2,3}. The cDNA sequence reported here establishes that the A chain does not arise from the same precursor polypeptide as the B chain of PDGF. The data presented here do not, however, exclude the possibility that *c-sis* encodes the A chain of PDGF, as it could be generated by differential splicing. It is unknown whether endothelial cell-derived PDGF-like mitogen is also an A/B chain mixture, or whether endothelial cells even synthesize the A chain of PDGF. It is possible that the endothelial mitogen is a B-chain homodimer, as has been suggested for porcine PDGF²⁷. The eventual generation of complete PDGF A-chain and B-chain cDNA clones may elucidate both the functional interactions between these polypeptides and the potential issue of coordinate expression. Finally, the 5' cDNA sequence information presented here should assist in locating the true 5' end of the human *c-sis* gene and, with it, possible sequences that regulate endothelial cell PDGF expression *in situ*^{8,28}. Such information may be critical in understanding the role of endothelium in pathological processes.

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Light-regulated and organ-specific expression of a wheat Cab gene in transgenic tobacco

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Many of our most important crop plants are monocotyledons, including wheat, corn, rice and barley. No routine transformation system for monocotyledons has been reported, such as the Ti-mediated gene transfer system for dicotyledons facilitated by *Agrobacterium tumefaciens*¹⁻³. Indirect evidence suggests that Ti-plasmid DNA is transferred into and expressed in *A. tumefaciens*-infected wound tissues of plants from Liliaceae and Amaryllidaceae^{4,5}, but these observations have not been extended to monocotyledons of greatest agricultural importance. Regeneration of monocotyledons is usually blocked at the callus stage, further complicating the possibility of exploring the regulated expression of their genes, and thus preventing identification of the regulatory domains of monocotyledonous genes in a homologous nuclear background. To circumvent these difficulties, we investigated whether monocotyledonous genes can be expressed and correctly regulated in dicotyledons. We have introduced a wheat gene (*whAB1.6*)⁶ encoding the major chlorophyll *a/b* binding protein (Cab) of the light-harvesting complex into the genomes of tobacco (*Nicotiana tabacum* SR1) and petunia (*Petunia hybrida*) via a Ti-DNA-mediated gene transfer system which allows the transformed cells to regenerate into whole plants⁷. Here we report for the first time the light-regulated and organ-specific expression of a monocotyledonous gene in transgenic dicotyledonous plants.

A 6.5-kilobase (kb) wheat genomic fragment containing the Cab gene (*whAB1.6*) was ligated into the *Cla*I site of the vector pMON145 (Fig. 1)⁸ and the construct was mobilized into a 'disarmed' *A. tumefaciens* derivative (GV3111SE)⁷. After co-cultivation of *A. tumefaciens* and protoplasts of *N. tabacum* SR1, transformed cells were identified by their resistance to kanamycin at 100 µg ml⁻¹ (ref. 1). Plants were regenerated from transformed calli according to Marton *et al.*⁹.

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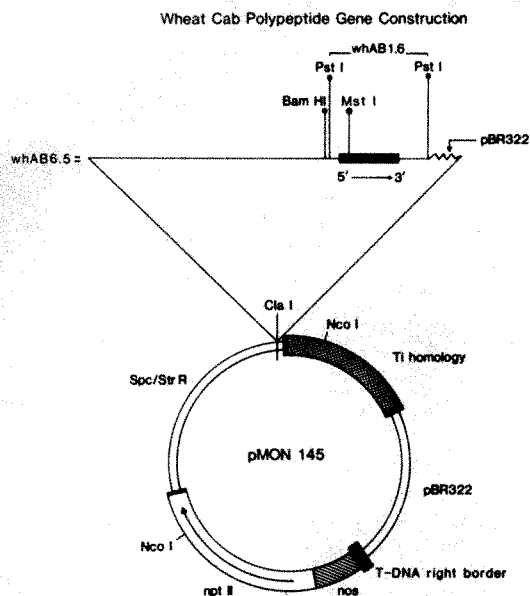


Fig. 1 Wheat Cab gene construction used for transformation. An 8.7-kb wheat genomic fragment containing the Cab gene, *whAB1.6* (ref. 6), was subcloned into pBR322 to yield the construct *whAB8.7*. Digestion of *whAB8.7* with *Cla*I releases a 6.5-kb fragment containing the entire wheat Cab protein-coding sequence, 4.4 kb upstream of the transcription start site, 800 bp beyond the stop codon and 250 bp of pBR322 at the 3' end. The 6.5-kb *Cla*I fragment was inserted into the *Cla*I site of pMON145, which has been described in detail elsewhere⁸. pMON145 contains the *NOS-NptII* fusion gene, a region homologous to *A. tumefaciens* Ti-DNA, a region of pBR322, spectinomycin (*spc*) and streptomycin (*str*) resistance genes. It also contains a 400-bp segment (*Pvu*I/*Sst*II) of the pTiT37 *Hind*III-23 fragment which includes the pTiT37 T-DNA right border⁸.

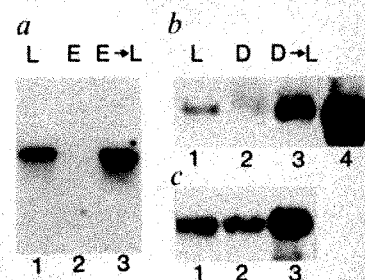
Methods. *Escherichia coli* strain LE392 was transformed with the *whAB6.5*/pMON145 plasmid construct, and colonies were selected for *spc* and *str* resistance. The *whAB6.5*/pMON145 construct was introduced by a triparental cross⁸ into a disarmed *A. tumefaciens* derivative (GV3111SE) containing a modified Ti-plasmid lacking phytohormone biosynthetic genes and TL-DNA right border⁷.

Northern blot analysis shows that in wheat the accumulation of Cab transcripts is strongly dependent on light (Fig. 2a). Using as a probe a labelled *Bgl*II/*Sma*I fragment, which encodes the carboxy-terminus of the mature Cab protein⁶, the amount of Cab transcripts in etiolated leaves is at least 20 times less than in green leaves. When etiolated wheat plants are transferred to the light for 24 h, leaf Cab messenger RNA increases to the amount that accumulates in leaves from plants grown in a normal light/dark cycle. The Northern blot analysis does not discriminate among transcripts from different members (at least seven) of the wheat Cab multigene family⁶.

To establish whether expression of *whAB1.6* is light-dependent, a gene-specific *Bam*HI/*Mst*I fragment was hybridized to the same RNAs and the products analysed by the *S*₁ nuclease assay¹⁰. The *Bam*HI/*Mst*I probe is 305 nucleotides long, and yields an *S*₁-resistant product of 169 nucleotides which includes the *whAB1.6* non-translated leader and extends 102 nucleotides into the Cab coding region. Figure 3a shows that this product is found only after light stimulation. We estimate that the *whAB1.6* transcript level increases at least 20-fold in going from the dark to the light.

To determine whether the wheat Cab gene is expressed in leaves of transgenic tobacco, and also whether it is light-regulated, we isolated RNA from leaves pooled from 10 individual transgenic tobacco clones. The plants were (1) grown under a light/dark, 16/8-h photoperiodic cycle, (2) transferred to the dark for 7 days and (3) transferred to the dark and then re-exposed to continuous light for 24 h. Blots of these RNAs were hybridized with the labelled *Bgl*II/*Sma*I fragment from *whAB1.6* (ref. 6), which does not cross-hybridize with the endogenous tobacco Cab mRNA in stringent conditions (Fig.

Fig. 2 Northern blot analysis of transcripts from wheat and transgenic tobacco leaves. *a*, Total RNA (10 µg) from wheat leaves of plants grown in the light (L) for 8 days in a 16/8-h light/dark cycle (lane 1), etiolated (E) plants grown in the dark (lane 2), or etiolated plants transferred to the light (E→L) for 24 h (lane 3). *b*, RNA (10 µg) from leaves of transgenic tobacco plants grown in a 16/8-h light/dark cycle from the callus stage (lane 1), young plants grown in the dark for 7 days (lane 2) and young plants transferred from the dark to the light (D→L) for 24 h (lane 3). Lane 4 shows a wheat leaf RNA (10 µg) control included in the tobacco analysis. *c*, Poly(A) RNAs were prepared¹¹ from leaves of transgenic tobacco plants. Lanes 1–3 are the same as lanes 1–3 in *b*. Each lane contained 1 µg of poly(A) RNA.



Methods. Total RNA was isolated from either wheat or tobacco leaves using the guanidinium thiocyanate procedure¹⁹, and Northern blots were analysed according to Carmichael and McMaster²⁰. The blots for *a* and *b* were hybridized to a ³²P-labelled *Bgl*II/*Sma*I fragment (10⁸ c.p.m. per µg) of 383 bp and encoding the carboxy-terminus of the Cab protein⁶. Hybridization was performed in 50% formamide, 2×Denhardt's, 50 mM NaPO₄, and 5×SSC, 100 µg ml⁻¹ calf thymus DNA at 46 °C. In these conditions the fragment does not hybridize with tobacco RNA (see Fig. 4a, lane 7). The blot for *c* was hybridized to a nick-translated probe containing the coding region of *NptII* in the same conditions.

Methods. Total RNA was isolated from either wheat or tobacco leaves using the guanidinium thiocyanate procedure¹⁹, and Northern blots were analysed according to Carmichael and McMaster²⁰. The blots for *a* and *b* were hybridized to a ³²P-labelled *Bgl*II/*Sma*I fragment (10⁸ c.p.m. per µg) of 383 bp and encoding the carboxy-terminus of the Cab protein⁶. Hybridization was performed in 50% formamide, 2×Denhardt's, 50 mM NaPO₄, and 5×SSC, 100 µg ml⁻¹ calf thymus DNA at 46 °C. In these conditions the fragment does not hybridize with tobacco RNA (see Fig. 4a, lane 7). The blot for *c* was hybridized to a nick-translated probe containing the coding region of *NptII* in the same conditions.

4a, lane 7). Figure 2b demonstrates that the wheat Cab gene is expressed in light-grown tobacco leaves at a level at least 4–10-fold higher than in leaves of dark-adapted plants (compare Fig. 2b, lanes 1, 2, and Fig. 4b, lanes 1, 2). On re-illumination, the wheat Cab mRNA level is often more than double the level found in plants grown in a 16/8-h light/dark photoperiodic cycle. The reason for this mRNA overproduction is being investigated. The wheat Cab mRNA level is reduced 4-fold in tobacco plants transferred to the dark and then increases 10-fold when plants are re-illuminated (Fig. 2b). To determine whether the reduction of the wheat Cab transcript level in the dark is selective, we prepared poly(A) RNAs¹¹ and analysed the mRNA levels for the chimaeric gene comprised of the nopaline synthase (*NOS*) promoter fused to the neomycin phosphotransferase (*NptII*) coding sequence (*NOS-NptII*). Figure 2c shows that the *NOS-NptII* transcript is indeed present in the dark-adapted plants and in plants returned to the light. The *NOS-NptII* gene has been demonstrated to be constitutively expressed in all tissues of plants which are kanamycin-resistant¹², including those transferred to the dark (F.N., unpublished results).

The Northern blot analyses demonstrate the presence of the wheat Cab transcript in transgenic tobacco leaves and that it is the same size (1,100 nucleotides) as in wheat. Furthermore, the wheat Cab mRNA can be recovered in the tobacco poly(A) RNA fraction (data not shown). These results suggest that the wheat Cab transcript is correctly processed and polyadenylated. To ascertain if accurate transcription initiation also occurs, transcripts from transgenic tobacco were mapped by *S*₁ nuclease protection. A 5' end-labelled *whAB1.6* *Bam*HI/*Mst*I fragment, described above, was hybridized with total leaf RNA from both light- and dark-grown plants, and the products were analysed alongside those obtained using wheat leaf RNA. A 169-nucleotide *S*₁-resistant product is obtained with either the wheat or the transgenic tobacco leaf RNAs (Fig. 3b). We conclude that transcription of the wheat Cab gene begins correctly in tobacco, and confirm with the *S*₁ nuclease results that the level of the wheat Cab transcript is also controlled by light in the transgenic plants. The transcripts are present in transgenic tobacco at ~10–25% the level found in wheat leaves (Fig. 3, compare lanes 1, 3, 5). These values do not reflect the absolute expression level of the wheat Cab gene because its copy numbers in the transgenic tobacco plants have not been determined. In contrast, the *NOS*-

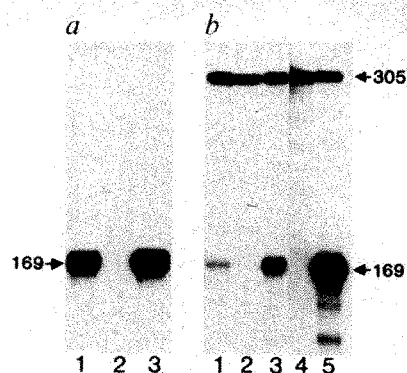


Fig. 3 S_1 nuclease analysis of light-regulated wheat Cab transcripts in wheat and transgenic tobacco. *a*, S_1 products using wheat RNAs. The same RNAs (10 μ g per lane) for lanes 1, 2 and 3 were used as described in Fig. 2a legend. *b*, S_1 products using transgenic tobacco RNAs. Lanes 1–3 are analogous to the lanes in Fig. 2b. Lane 4 shows hybridization of the *Bam*HI/*Mst*I fragment to untransformed tobacco RNA, and lane 5, a wheat leaf RNA control reaction. Each lane contained 10 μ g RNA. The band of high relative molecular mass (M_r) (305 nucleotides) corresponds to the undigested probe. The S_1 nuclease-resistant band at 169 nucleotides is caused by hybridization of the probe to the *whAB1.6*-specific transcript and the low- M_r bands (lane 5) represent hybrids between the probe and transcripts of other wheat Cab genes that show limited sequence homology to *whAB1.6* in the non-translated leader RNA region⁶.

Methods. RNAs were prepared from wheat and tobacco as described for Fig. 2. A 5'-end-labelled *Bam*HI/*Mst*I fragment from *whAB1.6* (ref. 6) was hybridized with 25 μ g of total RNA in 40 mM PIPES (pH 6.4), 1 mM EDTA, 0.4 M NaCl, 80% formamide. Samples were incubated for 4 h at 50 °C, digested with 30 units of S_1 nuclease (BRL) at 37 °C, in 0.3 M NaCl, 4.5 mM ZnCl₂, 5 mM sodium acetate and 20 μ g ml⁻¹ salmon sperm DNA¹⁰, and extracted with phenol/chloroform and ether. S_1 nuclease-resistant products were precipitated with 25 μ g of *E. coli* transfer RNA and aliquots were analysed on 8% acrylamide gels.

NptII transcripts can be detected only in the poly(A) RNA fraction (Fig. 2), which requires a ~100-fold enrichment step.

whAB1.6 is active in wheat leaves, requires light for activation (Fig. 3a) and is not expressed in roots⁶. To establish if the wheat Cab gene construct also contains sufficient information for organ-specific expression in the transgenic plants, RNAs were isolated from different organs of transgenic tobacco. We found that the wheat Cab gene is expressed predominantly in tobacco leaves by Northern blot analyses (Fig. 4a) and by S_1 nuclease protection assays (data not shown). In some RNA preparations the wheat Cab mRNA can be detected in tobacco stems at a lower level (~5–10-fold) than in leaves; expression continues to be light-regulated (Fig. 4b). We have also transferred the wheat Cab gene into *P. hybrida* and obtained accurate transcriptional initiation and light-regulated and organ-specific expression (data not shown).

Our results demonstrate that a wheat Cab gene is accurately transcribed and selectively expressed in transgenic tobacco and petunia. This is the first report of regulated expression of any Cab gene in transgenic plants. Our observations indicate that the dicotyledonous nuclear transcription apparatus recognizes the relevant regulatory sequences of the wheat Cab gene, and that the necessary information for gene activation is contained within a wheat genomic fragment which begins 4.4 kb 5' to the transcription initiation site and extends 850 base pairs (bp) beyond the Cab protein translation stop codon. Furthermore, the wheat Cab transcripts are polyadenylated (data not shown) and stable in tobacco and petunia cells.

A zein gene from maize is expressed in sunflower calli¹³, although it is not known whether organ-specificity of the zein gene will be retained in regenerated plants. Our finding that the wheat Cab gene functions with specificity in two dicotyledonous plants suggests that at least some dicotyledonous genes may show regulated expression in monocotyledons.

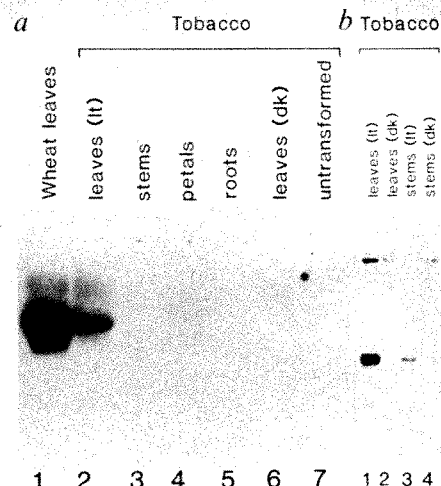


Fig. 4 Organ-specific expression of the wheat Cab gene in tobacco. *a*, Northern blot analysis. Lane 1, wheat leaves; lanes 2–5, leaves, stems, petals and roots, respectively, from transgenic tobacco plants grown under a 16/8-h light/dark photoperiod; lane 6, leaves of transgenic tobacco plants transferred to the dark for 7 days; lane 7, leaves of untransformed tobacco plants. Each lane contained 10 μ g RNA. *b*, S_1 nuclease protection assays. Lanes 1, 3, leaves and stems of transgenic tobacco grown under a 16/18-h light/dark photoperiod; lanes 2, 4, leaves and stems of transgenic tobacco transferred to the dark for 7 days. Each lane contained 10 μ g RNA.

Methods. RNAs were extracted from different plant organs¹⁹ and analysed by Northern blot hybridizations²⁰ or S_1 nuclease protection assays¹⁰. See Figs 2 and 3 legends for details.

The *cis*-acting sequences that control the wheat Cab gene have not been characterized. That the regulatory sequences of the wheat Cab gene functions with apparent fidelity in tobacco and petunia renders their identification feasible by combining *in vitro* mutagenesis and the *A. tumefaciens*-mediated gene transfer system, as has been done for the genes encoding the ribulose 1,5-bisphosphate carboxylase small subunit of pea (*rbcS*)^{14,21} and the 35S promoter of cauliflower mosaic virus¹². Our immediate goal is to compare the DNA sequences used for the light-dependent expression of the Cab¹⁵ and *rbcS* (refs 16, 17) genes. In peas the accumulation of Cab and *rbcS* transcripts begins at different red-light fluencies¹⁸, which suggests that the genes encoding key components of the chloroplast photosynthetic machinery are selectively responsive to light and regulated independently during shoot development.

This work was supported by a grant from Monsanto. G.L. was supported in part by a NIH postdoctoral fellowship (GM-08421) and F.N. is on leave from the Institute of Plant Physiology, Biological Research Center, Hungarian Academy of Science, Szeged, Hungary. We thank Sue Love for technical assistance, G. Morelli for helpful discussion and Wendy Roine for assembling the manuscript.

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المركز الدولي للبحوث الزراعية في المناطق الجافة

International Center for Agricultural Research in the Dry Areas

FOOD LEGUME CROP MICROBIOLOGIST

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2. To work closely with the agronomists and crop-physiologists in the FLIP and Farming Systems Program in studying the biological nitrogen fixation in field experiments evaluating novel production techniques and genotypes and long term rotational effects.
3. To strengthen contacts with national programs and help to enhance their efforts on symbiotic nitrogen fixation studies. Towards this end, to conduct special training programs in rhizobiology and to participate in the general food legume residential training courses.
4. To establish effective collaboration with other Centers of Excellence to augment the local rhizobiological research effort at ICARDA.

QUALIFICATIONS

A Ph.D in microbiology with at least 5 years experience of research in rhizobiology. Specialisation in the field of *Rhizobium* ecology would be desirable. Experience of working in developing countries with lentil, faba bean or chickpea would be considered desirable, and previous involvement in training activities would be useful. An essential prerequisite is competence in spoken and written English, and a knowledge of Arabic and/or French would be valuable.

CONDITIONS OF APPOINTMENT

The candidate would be based in Aleppo, North Syria and would be responsible to the Leader of the Food Legume Improvement Program. The candidate is entitled to an international tax-free salary based on background and experience; use of car; allowance towards housing; annual home leave; non-contributory medical insurance scheme; pension fund.

APPLICATION

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Applications should be received by 30 September 1985

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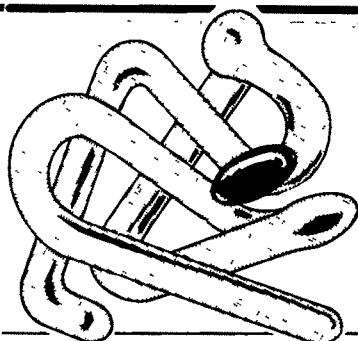
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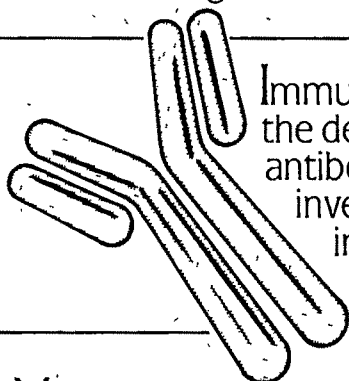
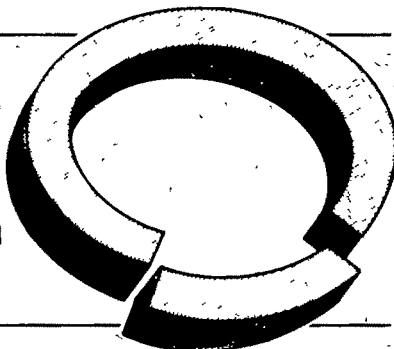
Applicants should have experience in device fabrication and low temperature physics. The appointment is for three years. Applicants should send a curriculum vitae and names of two referees to Dr N E Booth, Department of Nuclear Physics, Keble Road, Oxford, telephone (0865) 59911, as soon as possible.

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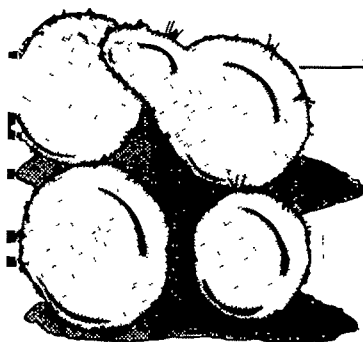
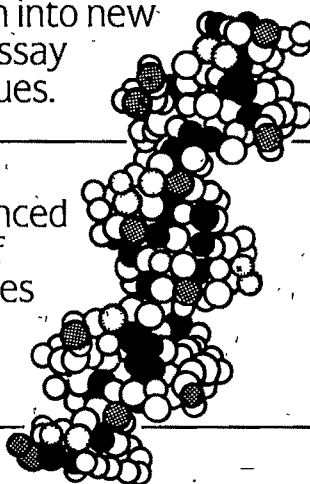
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Molecular geneticists experienced in gene identification, design of synthetic oligonucleotide probes and hybridisation techniques.



Cell biologists experienced in cell physiology, tissue culture and hybridoma - monoclonal antibody technology.

(7471)A

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Shown here is the range of scientific personnel we seek to join a new and dynamic research team involved in the development of nucleic acid - and immunoassay - based diagnostics for medical applications. Emphasis will be strongly based on the design of new diagnostics and the use of non-isotopic detection technology.

We are interested in applicants holding a good first degree or suitable equivalent qualifications, and those holding a Ph.D with, preferably, postdoctoral experience. Candidates should have a broad interest in the diagnostics field together with a flexible and adaptable approach to research areas geared to the market place. This will require a rare combination of both research and practical interest in the development of non-isotopic nucleic acid and immunoassay systems.

In return, we offer excellent technical facilities in attractive 'Greenfield' surroundings, opportunities for rapid career progression as the group expands, highly competitive salaries and conditions and a generous relocation package.

We intend to hold a series of regional meetings during September when selected applicants will be invited to find out more about our move into medical diagnostics.

Those who wish to take advantage of this exciting opportunity should send details of their career to date to Dr Trevor Twose, R & D Manager.

ICI Diagnostics Group

Stuart House, 50 Alderley Road,
Wilmslow, Cheshire SK9 1RE, UK.
Telephone. 0625 522022

VIROLOGY RESEARCH

Coopers Animal Health was formed by the merger of the worldwide interests of ICI and The Wellcome Foundation. The New Zealand company is located at Upper Hutt, 40 km north of Wellington and has a proud track record of success, in research and in the market place.

We wish to recruit suitably qualified virologists for the two positions of Head of Department and Research Scientist.

The Department is principally concerned with developing vaccines against viral diseases of veterinary importance in both large and small animal species. Therefore, in addition to a suitable background in virology, it would be of considerable advantage for an applicant to possess some additional applied research experience in these or related fields.

Successful applicants will also be encouraged to develop close working relationships with Government and other research establishments within New Zealand.

Whilst these are permanent positions, short or medium term appointments would be considered.

A competitive remuneration package, including an attractive salary which is subject to a regular merit review will be negotiated to reflect the significance of these positions to our research programme. Assistance will also be provided on travel and relocation expenses.

Please write enclosing a detailed curriculum vitae outlining your suitability for the position in which you are interested to —

John Rainey
Manager — Personnel Division



Coopers Animal Health NZ Limited

33 Whakatiki Street
Upper Hutt
New Zealand

(W2138)A

UNIVERSITY OF EAST ANGLIA Norwich LECTURESHIP IN SEDIMENTOLOGICAL GEOCHEMISTRY

Applications are invited for a lectureship in Sedimentary Geochemistry in the School of Environmental Sciences, starting from a date to be agreed between 1 January and September 1986. Salary will be on lecturer scale £7,520 — £14,925 p.a. (under review) plus U benefits.

Applications (three copies) which should contain a full curriculum vitae including exact date of birth, together with the names and addresses of three persons to whom reference may be made, should be lodged with the Establishment Officer, University of East Anglia, Norwich NR4 7TJ (telephone 0603 56161 ext 2126) from whom further particulars may be obtained, not later than 14 October 1985. No forms of application issued.

(7447)A

MOLECULAR BIOLOGIST

Tenure-track position at the assistant professor level for molecular biologist. Applicant must have at least 2 years postdoctoral experience and demonstrated accomplishment in eukaryote gene expression studies. Expertise in recombinant DNA technology essential. Ph.D. or M.D. Send curriculum vitae, a statement of research interest, and the names and addresses of 3 references to: Dr. Ralf Weichseleum, Professor Chairman, Dept. of Radiatic Oncology, Biological Sciences Division, The University of Chicago, Box 440, 5841 Maryland Ave., Chicago, IL 60637. An Affirmative Action Equal Opportunity Employer (NW1874)A

Faculty Position available, tenure-track, in molecular genetics, with a special interest in membranes. Applicants should have research background in recombinant DNA technology (preferably also in somatic cell genetics) and working knowledge of membrane proteins. M.D. and/or Ph.D. in appropriate field required. Successful candidate will hold tenure-track appointment (Assistant or Associate Professor, depending upon qualifications) in Department of Physiology and Molecular Biophysics at Baylor College of Medicine. Joint appointments in relevant academic departments also possible. Laboratory space and basic equipment provided and initial laboratory support available. Successful candidate will devote majority of his/her effort to establishing and maintaining an independent research program related to the main interests of the Department. The Department is building a strong program in membrane biophysics with expertise in electrophysiology, membrane biochemistry, reconstitution, and modelling. **Send vita, a brief description of past and present research experience, and names of three or more references to**

Dr. A. M. Brown, Incoming Chairman,
Physiology and Molecular Biophysics Department



Baylor College of Medicine,
One Baylor Plaza,
Houston, Texas 77030.

An Equal Opportunity A/A Employer

(NW58)A

UNIVERSITY OF ARIZONA POSTDOCTORAL POSITIONS A NUTRITIONAL BIOCHEMIST

is needed to study selenium metabolism using perfusion techniques and HPLC. Candidates should have a PhD in biochemistry or nutritional biochemistry, with background in metabolism.

A MOLECULAR BIOLOGIST

is needed to study the structure and regulation of glutathione peroxidase. Candidates should have a PhD in molecular biology, biochemistry or a related field, and experience with recombinant DNA technology.

Send resume and three letters of recommendations by 20 September to: Dr Roger A Sunde, Dept of Nutrition and Food Sciences, University of Arizona, Tucson, AZ 85721.

An Equal Opportunity /
Affirmative Action employer.
(NW161)A

FACULTES UNIVERSITAIRES NOTRE-DAME DE LA PAIX A NAMUR

DEPARTMENT OF BIOLOGY

1) ASSISTANT PROFESSOR POSITION

The candidate must be acquainted with plant biotechnology and specialized in protoplast fusion. He will have to develop the research in this field and will have some teaching courses in plant physiology and related area.

2) ASSISTANT OF HIGH QUALIFICATION

The candidate will have a minimum of a Ph.D. in the field of Botany.

He will develop the research in plant ecology in association with the laboratory of ecology of soil water.

He will be in charge of some courses in plant ecology.

Applications forms should be sent to the Recteur of the Facultés Universitaires, rue de Bruxelles 61, B-5000 NAMUR, BELGIUM. Closing date for application: 30 September 1985.

(W206)A

CENTER FOR ADVANCED RESEARCH IN BIOTECHNOLOGY

The University of Maryland and the National Bureau of Standards have jointly established the Center for Advanced Research in Biotechnology (CARB) to provide a unique forum for collaborative research among academic, government and industrial scientists. CARB's research facility is being constructed at the Shady Grove Life Science Center in Rockville, Maryland in close proximity to the NIH, the FDA, the USDA and at the heart of one of the nation's major centers of commercial biotechnology development.

CARB will initially focus on establishing what is expected to be the world's finest facility for the determination and analysis of the structure of macromolecules. The goals of CARB's research are to radically reduce the time and effort required to determine the atomic structure of proteins and to effectively model and predict their properties. This focused thrust will help build the foundation for the rapidly emerging field of protein engineering.

Toward this end, CARB will integrate a multidisciplinary team of scientists and engineers with state-of-the-art instrumentation and facilities, some of which will truly be one of a kind. CARB will draw upon the considerable expertise at NBS in physical and chemical measurement technology and will have access to unique research facilities including the research reactor's neutron diffraction facility and NBS's new CYBER 205 supercomputer. CARB will house scientists from the University of Maryland and the National Bureau of Standards as well as a large population of visiting scholars and industrial fellows, postdoctoral investigators and graduate students.

CARB is seeking **SENIOR RESEARCH SCIENTISTS** to staff research programs in the following areas. Senior Research Scientists should have an established research record in relevant disciplines and a demonstrated ability to obtain research support. Successful candidates will generally qualify for joint, tenure-track appointments in an appropriate department of the University of Maryland. Salary and rank will be commensurate with training and experience.

RESEARCH PROGRAMS

PROTEIN CRYSTALLIZATION has as its goal the development of general methodology and instrumentation for the routine and rapid growth of high-quality, single crystals of macromolecules and macromolecular complexes suitable for x-ray and neutron diffraction analysis. CARB will strive to develop a science of macromolecular crystallization from detailed studies of lattice interactions in protein crystals and development of methods for the real-time analysis and control of crystal growth. Novel application of site-directed mutagenesis for the preparation of heavy atom derivatives will be investigated. Methods for the growth of two-dimensional crystals will also be developed including monolayer and epitaxial approaches. In addition, the solid-state properties of protein crystals will be investigated.

X-RAY AND NEUTRON DIFFRACTION will include projects in all aspects of the crystallographic process with a target of reducing the time required to solve the structure of a new protein to a matter of weeks. Projects will include development of improved area detector systems and software, development of a tunable x-ray source for anomalous scattering methods, and development of automated map-fitting tools and refinement methods.

HIGH-FIELD 2D NMR will focus on determining the solution structure of macromolecules and will include instrument development work (1500 MHz) with particular emphasis on automated data reduction and resonance assignment.

ELECTRON MICROSCOPY will focus exclusively on three-dimensional studies of macromolecules and will include development of a scanning tunneling microscope for these applications.

MOLECULAR MODELING will include applications of supercomputers, multiprocessors and expert systems to molecular modeling development of an extensive macromolecular structure database for protein engineering and molecular graphics.

For additional information or to submit a resume contact:

Kevin M. Ulmer — Director
Center for Advanced Research in Biotechnology
University of Maryland
3300 Metzgerott Road, Adelphi,
Maryland 20783
(301) 853-3601



The University of Maryland and the National Bureau of Standards are Equal Opportunity/Affirmative Action Employers.

(NW155)A

THE UNIVERSITY OF MARYLAND

The Sandoz Institute for Medical Research Gower Place, London WC1

The Sandoz Institute for Medical Research is concerned with basic research on drugs which affect the nervous system. A multidisciplinary group of about 50 scientists and technicians are employed at the institute, which is situated within University College London. The institute

staff have close links with research groups within the University, and there are ample opportunities for collaboration. Applications are invited for two post-doctoral fellowships tenable for one year in the first instance, with a possibility of continuation for one further year.

1. Neuroimmunology

The appointee will be involved in the generation and exploitation of monoclonal antibodies to membrane receptors, under the direction of Dr J. N. Wood.

As part of this work, methods to generate monoclonal antibodies of predetermined specificity will be developed.

2. Sensory neurophysiology

The project will be concerned with the relationship between sensory neuropeptides and the regulation of spinal reflex excitability. This is a joint project between the Sandoz Institute and the Cerebral Functions Research Group at University College London directed by Professor P. D. Wall. The Post-Doctoral Fellow will be based at the Sandoz Institute.

but will be expected to collaborate actively with Professor Wall's group. The project will involve analysis of the peptide content of different types of sensory nerve, study of the transport of antibodies within sensory neurones and of the physiological effects produced, and investigation of the effects of putative sensory mediators on spinal reflexes.

Applicants should have experience in immunocytochemistry and radioimmunoassay, as well as a strong interest in neurophysiology and a willingness to participate in neurophysiological experiments. The salary range for both positions will be £10,000-£12,000 p.a., depending on age and experience.



Applicants should send a full curriculum vitae, and the names of two referees to Miss A. Giles, Sandoz Institute for Medical Research, 5 Gower Place, London WC1E 6BN. Closing date: 30th September 1985.

(7440)A

UNIVERSITY OF EDINBURGH DEPARTMENT OF BACTERIOLOGY

Virus Diagnostic Laboratory RESEARCH FELLOW

Applications are invited from a suitably qualified Postdoctoral Graduate in Biochemical, or Biological Sciences, to help in the development of a reference laboratory service for the diagnosis of infection with human T lymphotropic viruses.

The candidate would be expected to develop methods for the measurement of antibody as well as methods for the detection of virus.

The post is for one year with possibility of extension for a further year. Salary will be on the grade research scale (£7,520 — £12,000) with placement according to age and experience.

Applications by letter, including CV and the names of two referees should be sent to the Personnel Office, University of Edinburgh, South Bridge, Edinburgh EH1 1SU, not later than 12th September 1985.

Further particulars can be obtained from Dr J. F. Pleather and Dr E. Edmond, Department of Bacteriology, Medical School, Teviot Place, Edinburgh EH8 9AG (Tel 031 1011 Ext 2484 or 2251).

Please quote reference No 527 (7459).

Protein Chemist

American Cyanamid requires the services of a Protein Chemist at the Ph.D. level to assist in developing our biotechnology program at the Agricultural Research Division in Princeton, N.J.

The remuneration will be competitive, commensurate with applicant's experience.

Interested candidates are invited to submit a full curriculum vitae, a list of publications, and the names and addresses of three references to the Employment Office.



American Cyanamid Company

Agricultural Research Division
P.O. Box 400
Princeton, NJ 08540

An Equal Opportunity Employer M/F/H

No Agencies Please

(NW157)A



University of Massachusetts Medical School interdepartmental program in Environmental Health Sciences has available a tenure-track position in either Pharmacology or Pathology. Preferred areas of general research interest include Mutagenesis/Carcinogenesis or Inhalation Pulmonary Toxicology.

The University of Massachusetts Medical School seeks to recruit a tenure-track faculty member to join several of its faculty in continuing to develop the above interdepartmental program. The appointment may begin as early as the fall of the 1985-86 academic year. The appointee will be accorded considerable institutional resources and be expected to pursue an independent research program and to participate, in the context of an interdepartmental program, in service, medical education, and/or the education of graduate students in an institutional Ph.D. program.

Position Requirements: Appointment of a senior, established investigator at the level of Associate Professor or Professor is preferred, although exceptional candidates for Assistant Professor will be considered. The applicant should have the Ph.D. or equivalent, such as the M.D. or D.V.M. with appropriate research experience. Preference will be given to an investigator with an established program. The appointee's interest should fall within the context of the areas cited above and be relevant to the broad discipline of environmental-toxicologic pathology or Pharmacotoxicology, including the pharmacokinetics and metabolism and mechanisms of mutagenic/carcinogenic effects of drugs and environmental chemicals.

Deadline: General inquiries or letters of application (including a curriculum vitae and a list of three references) should be sent before September 15, 1985 to: Neal C. Brown, Professor & Chairman, Department of Pharmacology. (NW154)A

University of Massachusetts Medical School

55 Lake Avenue North
Worcester, MA 01605

An affirmative action/equal opportunity employer

FU

BERLIN

Freie Universität Berlin — Germany —
Invites applications for a position as

Professor in Microbiology (C3)

Preference will be given to candidates with a strong research record in microbial physiology and/or biochemistry. The applicant is expected to conduct independent research and to contribute to the teaching programme of the department, including undergraduate and graduate courses. A high academic qualification and teaching experience are required.

Send curriculum vitae, including a synopsis of past research and teaching within 6 weeks to

Freie Universität Berlin — Germany —
Fachbereich Biologie,
Grunewaldstraße 34 a, 1000 Berlin 41.

(W2140)A

**THE MACAULAY INSTITUTE
FOR SOIL RESEARCH
DEPARTMENT OF SOIL FERTILITY**



HIGHER SCIENTIFIC OFFICER

Applications are invited for appointment to a post of Higher Scientific Officer in the Department of Soil Fertility. The appointee will be required to study chemical and physiochemical factors which affect nutrient supply in the soil types prevalent in North Britain in relation to phosphate and the development of soil acidity. The successful candidate would be expected to devote part of his/her time to work on multi-disciplinary projects with crop physiologists and soil physicists within the Department, and in collaboration with other institutions.

Applicants should hold a First or Upper Second Class Honours Degree in Chemistry (with emphasis on analytical or physical chemistry), and preferably a PhD in Soil Chemistry. A minimum of 2 years' relevant post-graduate experience is required for appointment.

Salary £7,788 — £10,541 per annum, according to qualifications and experience. Non-contributory superannuation scheme operates. The Macaulay Institute for Soil Research is an Equal Opportunities Employer.

Forms of application and further particulars of the post may be obtained from The Secretary, The Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen, AB9 2QJ to whom they should be returned by 9 September 1985. Quote Ref. 85/9.

(7449)A

SENIOR POSITIONS IN CLINICAL/ MOLECULAR GENETICS MEHARRY MEDICAL COLLEGE

Two senior positions are available in the Department of Pediatrics as follows:

- I. A chair professorship endowed by the Kellogg Foundation. We are interested in making an appointment, as soon as possible, of an individual with professional interests in Clinical/ Molecular Genetics, although exceptionally talented individuals with research interests in other areas will be considered.
- II. Under the sponsorship of the March of Dimes — Birth Defects Foundation, Meharry Medical College will appoint a distinguished scientist in the area of clinical genetics and/or molecular biology in its Department of Pediatrics. The position is sponsored under a co-operative program between the March of Dimes — Birth Defects Foundation and Meharry Medical College.

For both positions, opportunity will be created for joint appointments in appropriate basic science departments. Interested individuals should hold the M.D. degree or MD/PhD and possess professional experience appropriate for appointment at the level of Associate or Full Professor. The selected individuals are expected to have demonstrated leadership qualities necessary to catalyze institution-wide interest and expansion of research in clinical/molecular genetics and/or research related to birth defects, and to participate in regular teaching/patient care and postgraduate training programs of the Department of Pediatrics and/ or appropriate basic science department.

Nominations or applications, containing curriculum vitae, names of four individuals who may be contacted for reference, and a summary of research interests or ongoing projects, should be sent to either: Ifeanyi J. Arinze, Ph.D., Chairman, Department of Biochemistry, or Festus Adebajo, M.D., Chairman, Department of Pediatrics, Meharry Medical College, Nashville, Tennessee 37208. Meharry Medical College is an equal opportunity/affirmative action employer.

(NW171)A

PLANT BREEDING INSTITUTE

is seeking to appoint two Geneticists and a Research Worker for a period of three years in a programme of 'Alien' gene transfers in wheat, funded by the Agricultural Genetics Company.

GENETICIST (CYT/218)

to take overall charge of and considerably extend an existing programme to introduce genes for novel endosperm proteins from related species into wheat and to explore the potential of such introduced genes in improving baking quality.

Appointment will be at Higher Scientific Officer/Senior Scientific Officer.

GENETICIST (CYT/219)

to lead a programme to transfer into wheat, from its wild relatives, new genes for resistance to a range of different diseases as well as genes of potential value in hybrid wheat production.

Appointment will be at Higher Scientific Officer/Senior Scientific Officer.

RESEARCH WORKER (CYT/220)

to provide support for post CYT/219 above.

Appointment will be at Scientific Officer.

Qualifications

For posts CYT/218 and CYT/219: First or Upper Second Class Honours degree in Genetics, Biochemistry or related subjects and at least two years postgraduate for appointment at HSO and four years for appointment at SSO.

For post CYT/220, an Honours degree in Genetics, Biochemistry or related subjects with an interest in cytogenetics and plant breeding and an awareness of electrophoretic techniques.

Salary: HSO — £7,788 — & £10,541 pa

SSO — £9,772 — £12,653 pa

SO£6,190 — £8,581 pa

Further particulars available on request.

Applications with cv and names and addresses of three referees for posts CYT/218 and CYT/219 and two referees for post CYT/220, should be sent to the Establishment Officer, Plant Breeding Institute, Maris Lane, Trumpington, Cambridge CB2 2LQ by 12 September 1985, quoting relevant reference number.

The Institute is an Equal Opportunities Employer. (7461)A



**Deutsches Krebsforschungszentrum/
German Cancer Research Center Heidelberg
Institute of Toxicology and Chemotherapy
POST DOCTORAL POSITION**

Applications are invited for a three year postdoctoral position to investigate the interaction of chemical carcinogens with DNA with molecular biological techniques.

The department of Dr M Wiessler is concerned with the action of chemical carcinogens on cellular macromolecules and on regulatory processes in the cell and whole organism.

The applicant should master methods in molecular biology to enable her/him to study the expression, in appropriate vectors, of synthetic oligonucleotides containing modified bases at defined positions.

The applicant should not be older than 32 years. The position is available immediately, the salary in the range of DM 44,000 - 51,000 per annum.

Applications should be sent to: Dr M Wiessler, Institut für Toxikologie und Chemotherapie, Deutsche Krebsforschungszentrum im Neuenheimer Feld 280 0900 HEIDELBERG, Tel 06221/4841 (W2142)A

**THE DEPARTMENT OF PATHOLOGY, NEW YORK STATE
College of Veterinary Medicine**

is offering a tenure track faculty position in veterinary pathology. Responsibilities will include participation in core curriculum teaching for veterinary students, rotational assignments to diagnostic pathology services, development of independent and collaborative research programs in experimental pathology, and supervision of post DVM graduate students.

Preference will be given to candidates possessing specialty Board-certification by the American College of Veterinary Pathologists and a demonstrated research background in toxicologic pathology or chemical carcinogenesis. Academic rank and salary will be commensurate with background and experience.

Appointment will be available 1 August 1985. Candidates should submit curriculum vitae, five letters of reference, and a statement of career goals to: Robert M Lewis, DVM, Chairman, Department of Pathology, New York State College of Veterinary Medicine, Cornell University, Ithaca, New York 14853.

Cornell University is an Equal Opportunity Employer/Educator (NW156)A

SENSORY NEUROBIOLOGIST

The Department of Neurobiology and Physiology at Northwestern University is seeking applications for a faculty position, at the level of Professor or Associate Professor in the general area of Sensory Neurobiology. The successful candidate will have a strong, well established program of research, preferably in developmental or cellular/molecular neurobiology dealing with one of the sensory systems.

The Department of Neurobiology and Physiology has strong, well-funded groups in Sensory Neurobiology and in Neuroendocrinology, both representing multidisciplinary approaches to these areas of Neuroscience. The Department currently administers a PhD program; two NIH training programs are centred in the department. The Department also has an undergraduate major leading to a BA, and a BAMS honours program for exceptional undergraduates.

Interested persons should submit a curriculum vitae and the names of three persons who can provide references to:

Dr. Albert I. Farbman,
Department of Neurobiology and Physiology,
Northwestern University,
Hogan Hall, Evanston, IL 60201.

Northwestern University is an Equal Opportunity/Affirmative Action Employer. (NW167)A

**UNIVERSITY OF LA
LAGUNA
CANARY ISLANDS**

**Announces a vacancy as
VISITING PROFESSOR IN
ASTROPHYSICS**

Applications are invited for a 1 year position as a visiting professor at the Departamento de Física de la Tierra y del Cosmos, with possible extension for a longer period.

This position involves teaching astrophysics to Undergraduates in physics (speciality of Astrophysics) and leading research groups with postgraduates. The candidates should have broad research experience and have directed groups in astrophysics. Research will be carried out at the Institut de Astrofísica de Canarias to which depend the International Observatories of "Teide" (Tenerife) and "Rogue de los Muchachos" (La Palma). Knowledge of the spanish language at an elementary conversational level is required. A higher level would be favourably considered. The salary will be 2.419.960 pesetas.

Applications must be accompanied by curriculum vitae and a list of publications. These must be submitted to EXCMO RECTOR University of La Laguna, La Laguna, Tenerife, before the 20th of October, 1985 (W2139)A

**ST GEORGE'S HOSPITAL
MEDICAL SCHOOL
(University of London)
TECHNICIAN 3**

required to join the established staff of the Electron Microscopy suite. The suite consists of transmission and scanning EM and freeze-fracture equipment and provides a service for pre-clinical departments.

Training will be given in EM techniques. Scale £5,399 — £6,325 pa plus London Allowance £730 pa. Further details and an application form available from the Personnel Officer, St George's Hospital Medical School, Cranmer Terrace, Tooting, London SW17 0RE (tel 01-672-1255 ext 4873). Closing date for applications 12 September 1985. Quote reference 13/85 (7473)A

**POSTDOCTORAL
POSITION
YEAST MOLECULAR
GENETICS**

available immediately to study the dsRNA viruses of *S. cerevisiae* with emphasis on the chromosomal genes which affect viral replication and gene expression. Attractive compensation package. Potential for academic appointment with the opportunity to develop an independent research program in 2-3 years. Please submit curriculum vitae and names of three references to Steve S. Sommer, MD, PhD, Department of Cell Biology, Mayo Foundation School of Medicine, Rochester, MN 55905.

An equal opportunity/affirmative action institution (NW163)A

**UNIVERSITY OF
CAMBRIDGE
Department of Pathology
POST-DOCTORAL
MOLECULAR BIOLOGIS**

Applications are invited for a postdoctoral position, tenable for 1 years from 1st October 1985, in a small group investigating immunology and molecular biology of schistosomiasis.

Candidates are sought with experience in molecular biology who would be primarily concerned with the preparation and detection of cDNA clones encoding and expressing antigens associated with the development of protective immunity but would also be free to follow other areas of current interest in molecular biology of schistosomiasis. Previous post-doctoral experience would be desirable, but is by no means essential.

Salary on postdoctoral scale dependent on age (£7,520 age 24 £9,390 age 28, under review).

Informal enquiries welcomed to A E Butterworth or Dr D W Taylor (Department of Pathology, Tennis Court Road, Cambridge CB2 1Q Telephone 0223-64631).

Formal applications, including curriculum vitae and the names of two referees, to Mr P Wilburn, Superintendent, Tennis Court Road Cambridge CB2 1QP (7463)A

**UNIVERSITY OF ILLINOIS
College of Dentistry
DEPARTMENT OF
ORAL ANATOMY
HEAD**

Individual of scientific attainment sought to head a department with research emphasis in function, morphology and teaching duties in gross, neuro and dental anatomy. Both DDS and PhD are desirable. Position available July 1, 1986.

Send curriculum vitae, publications and names of five references to Dr Dale R Eisenmann, Chairman of Search Committee, Department of Histology, University of Illinois at Chicago, PO Box 6998, Chicago, Illinois 60680. The University of Illinois is an Affirmative Action Equal Opportunity Employer. (NW170)A

**PRINCETON UNIVERSITY
GEOCHEMIST**

to participate in the development of models of trace metal cycling processes for incorporation in three dimensional ocean circulation and biological cycling models presently in development. Participation in measurement programs a possibility. Appointment will be to the Research Staff at the postdoctoral level or higher. Send résumé and 3 references to the Director, Geophysics Fluid Dynamics Program, Department of Geological and Geophysical Sciences, Princeton University, Princeton, NJ 08544. Princeton University is an equal opportunity affirmative action employer. (NW169)A

THE UNIVERSITY OF PAPUA NEW GUINEA

Port Moresby

SENIOR LECTURER/
SOCIATE PROFESSOR IN

DEPARTMENT OF FISHERIES

Applications are invited for the above position in the Department of Fisheries. The post falls vacant at the end of 1985. Applicants should have a B.Sc. in Fisheries or a related discipline. Preference will be given to candidates with both teaching and research experience in the discipline. The appointee shall be responsible for academic leadership in the Department of aquatic resources management in the Department, and should have proven expertise in the following areas: Fisheries Management, Economics/Fisheries Economics, Aquatic Resources Management. Experience in Fish Population Dynamics and Quantitative Methods in Fisheries could be an advantage.

Salary: Associate Professor K23,500 per annum plus gratuity; Senior Lecturer K21,255 per annum plus gratuity; Lecturer Grade 2 K19,405 per annum plus gratuity; Lecturer Grade 1 K17,555 per annum plus gratuity.

Conditions of Appointment The successful applicant will be normally offered a contract for a three year appointment ending at the end of a calendar year. The gratuity entitlement is based on 24% of salary earned and is payable in instalments or lump sum. It is taxed at a flat rate of 2%. In addition to the salaries quoted above, the main benefits include support for approved research; rent; accommodation; appointment of repatriation airfares for appointee and dependents; financial assistance towards the cost of transporting personal effects to and from PNG; 6 weeks annual recreation leave with home airfares available after each 18 months of continuous service; generous education subsidies for children attending schools in PNG or overseas; a salary continuation scheme for extended illness or disability. Applicants wishing to arrange secondment from their home institutions will be welcomed. The terms and conditions are under review.

Applications will be treated as strictly confidential and should include a full curriculum vitae, a recent passport photograph and the names and addresses of three referees. Applications should be forwarded to the Deputy Registrar (Staffing), University of Papua New Guinea, PO Box 90, University Post Office, Papua New Guinea, to reach him as soon as possible. Candidates in the UK could also send a copy of their applications to the Secretary General, Association of Commonwealth Universities (Appsts), 36 Gordon Square, London WC1H 0PF. (W2143)A

Please mention
nature
when replying to
these advertisements

NATURE CONSERVANCY COUNCIL

MARINE/COASTAL
ECOLOGIST

Higher Scientific Officer
13 month contract post

Applications are invited for the above contract post with the Chief Scientist Directorate, to be based in the Nature Conservancy Councils Great Britain Headquarters in Peterborough.

The post is jointly funded by British Petroleum and the NCC and will be for 13 months in the first instance but possibly extending to three years. The project will involve the planning, promotion, co-ordination and analysis of surveys of coastal and intertidal habitats by voluntary bodies, educational and amateur groups. The person appointed will be expected to travel widely throughout Great Britain and should have some experience in data handling.

Qualifications: Applicants should have a degree in biology, geography or a related subject and have at least 2 years relevant post graduate experience. A current driving licence is essential.

Salary: depending on qualifications and experience £7,788 rising to £10,541.

For an application form and further details of the post, please contact Miss B.A. Meredith, Personnel Officer, Nature Conservancy Council, Northminster House, Peterborough, PE1 1UA, to whom completed forms should be returned by 12th September 1985.

The Nature Conservancy Council is an equal opportunity employer. (7450)A

ST GEORGE'S HOSPITAL MEDICAL SCHOOL

(University of London)

DEPARTMENT OF BIOCHEMISTRY

Cancer Research Campaign
Mammalian Protein

Synthesis and Interferon
Research Group

JUNIOR POSTDOCTORAL POSITION IN CELL GROWTH REGULATION

A vacancy exists for a postdoctoral scientist to work on the regulation of cell proliferation by growth factors and interferons. Experience in tissue culture techniques and an interest in the control of protein synthesis or DNA replication would be an advantage. The post is available until December 1987 and must start on the first point of the Grade 1A scale (£7,520 plus £1,233 London Weighting).

Further details may be obtained from Dr Mike Clemens (telephone 01-672-1255 ext 4750). Application form available from the Personnel Officer, St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE. (tel 01-672-1255 ext 4873). Closing date 12 September 1985. Quote reference 15/85 (7474)A

Cell Physiologist (PhD)

elf bic
recherches

Elf Bio Recherches, a subsidiary of Elf Aquitaine and Sanofi, specialises in biotechnology. It is looking for a Cell Physiologist for its laboratory of animal cell physiology and fermentation. This laboratory (2 scientists and 3 technicians) forms part of the recombinant animal cell technology unit. The research activities of this group include the investigation of the role of physiological factors involved in the expression of genes cloned into animal cells. Candidates should have a profound knowledge of animal cell physiology. Experience in medium or large-scale tissue culture will be an advantage. Knowledge of French would be appreciated. Elf Bio Recherches R & D facilities are located in the Toulouse area, in Southern France. Curriculum vitae and list of publications should be sent to Mr Fabre quoting **Reference B/T 2018N**. (W2149)A

PA

71 bis, allées Jean-Jaurès, 31000 Toulouse, France.
Tel: Toulouse 63.70.63.

ELSEVIER PUBLICATIONS (Cambridge)

We are looking for an

EDITOR

for a monthly review journal in the biological sciences. The ideal candidate will have:

- Research experience in *either* some area of ecology or evolutionary biology. Applicants must be able to demonstrate a broad knowledge of current issues in population biology (This will be a new appointment), *or* some area of neuroscience. Applicants must be able to demonstrate a broad interest in clinical, systems and molecular neurobiology.
- Experience and ability in scientific editing or writing.
- Enthusiasm for explaining the latest advances in a rapidly changing field to an audience with a wide diversity of experience.
- Organisation skills, an eye for detail and the ability to work to deadlines.
- The position will ideally suit someone with post-graduate research experience, aged 30, or less.

The new journal will be published alongside our current successful line of monthly review journals in biomedicine, including *Trends in NeuroSciences* and *Trends in Biochemical Sciences*. The position is situated in our pleasant modern offices in Cambridge. The salary will be based in age and experience.

Please send your c.v. and relevant details to:

Dr J.D. Bousfield,
Elsevier Publications (Cambridge),
68 Hills Road, CAMBRIDGE CB2 1LA (7487)A

Harvard University
ASSISTANT PROFESSOR
CELLULAR AND DEVELOPMENTAL
BIOLOGY (CDB)

The Department of Cellular and Developmental Biology intends to make one and possibly more appointments at the assistant professor level effective July 1, 1986. Our department covers a broad range of topics in cellular biology and developmental/ molecular genetics. We are seeking outstanding candidates in these fields. We are particularly interested in possible appointments in the areas of vertebrate development, neurobiology, plant molecular biology, and cell biology. However, we will consider exceptional applicants in other fields as well. We encourage applications from women and minority groups. Applications should include curriculum vitae, reprints of publications, and a statement of research plans. Complete applications and three letters of recommendation, solicited by the applicant, should be received no later than October 31, 1985 by:

Diane Baldwin, Administrator
 CDB Search Committee
 Cellular and Developmental Biology
 The Biological Laboratories
 Harvard University
 16 Divinity Avenue
 Cambridge, MA 02138

Harvard University is an Affirmative Action/Equal Opportunity Employer (NW102)A

CELL BIOLOGY
AND PHYSIOLOGY
WASHINGTON UNIVERSITY SCHOOL
OF MEDICINE
ST LOUIS, MISSOURI

The Department of Cell Biology and Physiology, Washington University School of Medicine, St Louis, Missouri, is expanding its commitment to research in the following areas: (i) membrane protein function and biogenesis; (ii) assembly, function, and movement of intracellular organelles; (iii) cell motility and contractility; and (iv) cell-cell and cell-matrix interactions. Individuals who employ novel biophysical and recombinant DNA methodologies are especially encouraged to apply. Tenure track positions are available for successful candidates who will be expected to develop an independent research program and to participate in the training of medical and graduate students.

Please submit curriculum vitae and the names of three references to: Dr Philip Stahl, Department of Cell Biology and Physiology, Washington University School of Medicine, 660 South Euclid Avenue, St Louis, MO 63110.

An Affirmative Action / Equal Opportunity Employer.
 (NW158)A

ASSISTANT PROFESSOR
OF MICROBIOLOGY

The Department of Microbiology, University of Illinois, Urbana, invites applications for a full-time (9 months/year) tenure-track appointment at the assistant professor level. The position is available for August of 1986. Applications must have a Ph.D. and at least two years of postdoctoral experience. Preference will be given to applicants who have demonstrated research competence in molecular biology and who plan to work in the general area of microbial genetics and physiology. The appointee is expected to establish a vigorous research program, to participate in undergraduate and graduate teaching, and to supervise graduate students. Salary will be commensurate with education and experience.

Applicants should send curriculum vitae, a summary of future research interests, and the names and addresses of three references to: Dr. A. Salyers, Department of Microbiology, University of Illinois, 131 Burrill Hall, 407 S. Goodwin, Urbana, Illinois 61801 (217-333-7378). To receive full consideration applications should be received by November 15, 1985. Some interviews may be conducted before November 15, 1985. However, all applications that are received by that date will be given equal consideration. (The University of Illinois is an Equal Opportunity/Affirmative Action Employer).
 (NW134)A

GEOLOGICAL
ENGINEERING/
ENGINEERING
GEOPHYSICS

Tenure Track

Applications are invited from suitable qualified geologists and engineers to teach geological engineering classes at both the graduate and undergraduate level and to pursue research interests in either experimental or computational rock mechanics.

Applicants should be qualified at the Ph.D. Level and ideally should be eligible for registration as Professional Engineers. Appointment is likely to be made at the rank of assistant professor.

Letters of application naming three referees should be addressed to Professor M.J. Reeves, Search Committee Chairman, Department of Geological Sciences, University of Saskatchewan, Saskatoon, Sask., S7N 0W0, Canada. (NW162)A

UNIVERSITY OF LONDON
Institute of Neurology
POSTDOCTORAL
RESEARCH ASSISTANT

A postdoctoral research Assistant with research experience in psychopharmacology and related fields is required for an MRC-supported project on the behavioural functions of GABA — and benzodiazepine-sensitive brain pathways in rats. The present project will have another two years to run, from a date on or after October 1st 1985. Salary and superannuation on University scales.

Apply with CV and names of referees to Dr L J Herberg, Experimental Psychology Laboratory, Institute of Neurology, the National Hospital, Queen Square, London WC1N 3BG.

(7448)A

LABORATORY OF
MOLECULAR BIOLOGY

Genzentrum Munich/
 Martinsried

POST DOCTORAL
SCIENTIST

Student (holding a masters degree, aim: Dr rer, nat)
TECHNICIAN

Applicants should have experience or strong interest in: Molecular Biology, Gene cloning — sequencing, Immunology, Biochemistry. The successful candidate(s) will join a young team and enjoy to do science on the following subjects: Molecular characterisation of the transformed cell, control of proliferation in the normal and transformed cell, virus-cell interaction, cell differentiation.

The positions are available but may be delayed for suitable candidates. Please write enclosing CV and names of two referees to: Dr K H Westphal, Lab f, Mol, Biol, Genz, MPI/Biochemie, Am Klopferspitz, 8033 Martinsried, FRG
 (W2147)A

UNIVERSITY OF LONDON
CHAIR OF VETERINARY
MOLECULAR AND
CELLULAR BIOLOGY

at the Royal Veterinary College

The Senate invite applications for the above Chair which is newly established. Candidates should possess a veterinary, medical or biological science degree and a PhD or similar higher degree. A good knowledge of the techniques of biotechnology, proven research record relevant to veterinary science and experience in teaching are required. The person appointed will head an inter-departmental project team which will bring together a group from various departments of the College to use techniques of biotechnology which are vital to the development of veterinary science and medicine. This would be the only such inter-departmental group in a veterinary school and only such Chair in UK.

Salary within the Professor's range. The current minimum £19,303 including London Allowance.

Removal expenses may be reimbursed up to £1,400.

Intending applicants should obtain further particulars from the Academic Registrar (N), University of London, Malet Street, London WC1E 7HU before submitting applications (10 copies).

The closing date for receipt of applications is 20 September 1985. Potential candidates are invited to contact the Principal of the Royal Veterinary College. (7466)A

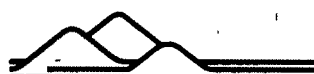
ROYAL PERTH HOSPITAL
Western Australia
GENETICIST/COMPUTER
PROGRAMMER/
BIOMEDICAL
STATISTICIAN

Applications are invited for a position suitable for a Post Doctoral Fellow, funded by the Western Australia Arthritis and Rheumatism Foundation and administered by the University of Western Australia. Duties will include maintenance and development of computer programmes used in genetic analysis including DNA sequencing. The applicant will join a group involved in N + MRC funded research on immunogenetics and will be expected to provide advice on statistics and maintain patient and population data bases relevant to arthritis and immunogenetics.

The appointment will initially be for one year with a probability of renewal. With relevant qualifications salary will commence at \$26,236 PA.

Enquiries and applications should be directed to: Dr R L Dawkins, Department of Clinical Immunology, Royal Perth Hospital, Wellington Street, Perth, Western Australia 6000.

Closing date September 20, 1985.
 (W2146)A



DERBYSHIRE COLLEGE
OF HIGHER EDUCATION

Research Assistant in Fern Ecology

(3 Years)

Applications are invited by 13th September 1985 from graduates with a good honours degree to study the establishment of ferns in woods near Derby. The successful applicant will be expected to register for a higher degree.

Salary: £5,181 to £6,405 per annum subject to revision

Application forms and further details from the Staffing Officer, Derbyshire College of Higher Education, Kedleston Road, Derby DE3 1GB.

(7472)A



POST VACANCY FOR A SCIENTIFIC PROGRAMMER IN ADDIS ABABA VACANCY INT/002/85

The International Livestock Centre for Africa, ILCA, has a vacancy for a Scientific Programmer at its headquarters in Addis Ababa. The successful candidate will have a basic animal science degree or equivalent, post graduate qualifications with at least five years of experience in computer science and proven qualities in undertaking all aspects of extraction, analysis and interpretation of major data sets in animal production and related fields. The main language is Fortran. Hardware involved are 2 HP3000 mini-computers and a network of micros. Experience with micros is also desirable, and an ability to liaise with a wide range of research workers essential.

This is an international staff position and the salary will be paid in US dollars. The initial contract will be two years renewable annually thereafter, subject to satisfactory performance and financial resources being available. Initial salary level will be determined by qualifications and experience. Moving and education allowances are paid and there are medical and retirement benefits.

Applications should include a current curriculum vitae and the names and addresses of three referees. Applications citing the reference number should be sent by 30th September 1985 to the Personnel Officer, International Livestock Centre for Africa, P.O. Box 5689, Addis Ababa, Ethiopia.

(W2150)A

UNIVERSITY OF CAMBRIDGE

Post-graduate/post-doctoral RESEARCH ASSISTANT NEUROENDOCRINOLOGIST

To join multi-disciplinary neuro-endocrine research group in MRC supported project to investigate the neural basis of photorefractoriness in seasonal breeding. Background in endocrinology/neuroscience preferable.

Post available autumn 1985 for three years. 1A salary scale.

Applications and further details: Mrs J Penton, Dept. of Anatomy, University of Cambridge, Downing Site, Camb CB2 3DY. Please arrange for 2 referee's reports to accompany application. (7454)A

ROYAL PERTH HOSPITAL Western Australia POST DOCTORAL FELLOW

At least one Post Doctoral Fellow is required to develop an aspect of several NH + MRC projects investigating the immunogenetics of autoimmune disease, utilising recombinant DNA technology. Some experience in molecular biology and immunogenetics is desirable. The appointment will be for one to three years. Salary will be based on NH + MRC scales commencing at A\$22,614 but with supplements as appropriate.

Enquiries and applications should be directed to: Dr R L Dawkins, Department of Clinical Immunology, Royal Perth Hospital, GPO Box X2213, Perth Western Australia 6001

Closing date September 20 1985
(W2145)A

JOHN INNES INSTITUTE SCIENTIFIC OFFICER

Applications are invited for a Scientific Officer to assist in a research programme aimed at the regeneration of crop plants from tissue cultures. This is a funded post and the appointment will be for approximately 18 months.

Applicants should have an honours degree in a biological science or HNC with relevant post-qualifying experience.

The appointment will be to the Scientific Officer grade, with salary on the scale £6,190 to £8,561. Non-contributory superannuation.

Applications, quoting reference AG/202, together with a full cv and the names of two referees should be sent to the Secretary, John Innes Institute, Colney Lane, Norwich, NR4 7UH. (7451)A

CAMBRIDGE MRC Laboratory of Molecular Biology POST-DOCTORAL SCIENTISTS

Applications are invited for post-doctoral positions of about 2 years' duration in microbial or molecular genetics. Experience in recombinant DNA techniques would be advantageous but training can be provided.

Salary in the range £8,920 — £14,925 pa. Starting date flexible but autumn 1985 preferred

Applications — including a full CV, a short statement of research experience and the names of two professional referees — should be sent by 12th September 1985 quoting reference MG/85 to the Assistant Director, MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH (7441)A

POSTDOCTORAL RESEARCH AND TRAINING OPPORTUNITIES

are available at the

CHEMICAL INDUSTRY INSTITUTE OF TOXICOLOGY

The Chemical Industry Institute of Toxicology (CIIT) offers a two-year post-doctoral research training program in toxicology for individuals who have recently earned a D V M M D. or Ph.D. in an academic discipline related to toxicology. Research programs stress elucidation of basic mechanisms of toxicity and often employ a multidisciplinary approach. Areas of interest include mutation and chemical carcinogenesis, pharmacokinetics and metabolism, teratogenicity, development of *in vitro* tests for toxicity, immunotoxicity, reproductive system toxicity, inhalation toxicology, epidemiology and quantitative risk assessment.

For more information contact: Coordinator of Training, Chemical Industry Institute of Toxicology, P.O. Box 12137, Research Triangle Park, NC 27709. (NW166)A

MARIE CURIE MEMORIAL FOUNDATION RESEARCH INSTITUTE POST DOCTORAL RESEARCH POSITIONS

to study the role of the cellular p53 oncogene in multi-stage carcinogenesis.

1. The role of p53 in SV40 and Adenovirus transformation. Reagents available include p53 deletion mutants that bind SV40T or Elb55k but are defective for cellular immortalization, point mutants that fail to bind the viral oncogene products and p53 antisense retroviral vectors.
2. Investigation of p53 mutants that exhibit enhanced activity in cellular immortalization assays.

These three year positions are funded on the University Lecturer scale.

Applications, C.V. and the names of two referees to Dr. J. Jenkins, MCMF Research Institute, The Chart, Oxted Surrey (088-388-2306). (7445)A



Athrofa Gogledd Ddwyrain Cymru
The North East Wales Institute
 of higher education

CONNAH'S QUAY, DEESIDE, CLWYD
RESEARCH DIVISION
POSTDOCTORAL RESEARCH
ASSISTANT BIOTECHNOLOGY
 (INITIALLY FOR ONE YEAR)

A Research Assistant is required to work on a joint SERC/industrial programme on the development of immunoassays for food analysis. Applicants should have a good honours degree and Ph.D., with experience of antibody preparation and immunoassays, especially ELISA. The appointment, initially for 12 months, will be on the Researcher B1 Scale (£7,548 — £10,251 pa).

Further details and application form from The Registrar, The North East Wales Institute, Connaah's Quay, Deeside, Clwyd. Tel: Deeside 817531 Extn. 254. Closing date for receipt of applications 7th September 1985. (7488)A

UNIVERSITY OF DUNDEE
DEPARTMENT OF BIOCHEMISTRY
POST-DOCTORAL
RESEARCH ASSISTANT

Applications are invited for the above SERC-funded position which forms part of a small group studying the mechanism of cofactor acquisition by molybdoenzymes in *E. coli*. The project involves the biochemical analysis of a series of mutants which are defective in molybdoenzyme biosynthesis. Enzymological and molecular genetic approaches are being employed. Persons with experience in either or both of these areas are preferred but other strong applicants will also be considered. Further information may be obtained from Dr D H Boxer (0382 23181, ext 4250). The position is available for up to three years starting as soon as possible.

Appointment will be on the Research & Analogous 1A scale currently £7,520 — £12,150 (under review) depending on qualifications and experience.

Please apply enclosing a curriculum vitae and the names of two referees to The Personnel Officer, The University, Dundee, DD1 4HN as soon as possible. Please quote Ref EST/75/85J. (7453)A

UNIVERSITY OF BATH
School of Biological Sciences

LECTURER IN
VEGETABLE CROP
PRODUCTION

(Three year contract with the possibility of renewal)

Applications are invited from plant physiologists with experience of field and/or protected vegetable crop production, including field experimentation. The successful candidate will be expected to develop a research programme which interacts with existing interests in one or other of the following areas: stress physiology, seed physiology, micropropagation, growth regulators.

The teaching duties will be concerned with the BSc Course in Horticulture and the MSc Course in Crop Production (Horticulture).

Salary within the range £7,520 — £14,925.

Further particulars and application forms may be obtained from the Personnel Officer, University of Bath, Bath BA2 7AY, quoting reference number 85/127.

Closing date for applications: 12th September 1985. (7443)A

*** * * SENIOR SCIENTIST * * ***

The W. Alton Jones Cell Science Center, Inc., Lake Placid, New York has an immediate opportunity for a young, innovative and experienced molecular biologist to direct independent research in the field of molecular and cellular biology.

We are seeking a dedicated and talented scientific professional with interest in cell biology and growth factors. The successful candidate will have a doctorate in molecular and cellular biology with at least 3 years postdoctoral experience. Candidates must have the ability to supervise others. Liberal benefits. Salary will commensurate with experience.

Applicants are requested to submit complete curriculum vitae and to have three letters of academic references forwarded by October 1, 1985 to:

Personnel Manager
W. ALTON JONES CELL SCIENCE CENTER,
10 Old Barn Road
Lake Placid, New York 12946

AN AFFIRMATIVE ACTION/EQUAL OPPORTUNITY EMPLOYER
 (NW151)A

UNIVERSITY OF
EAST ANGLIA
NORWICH
THERMOPHYSICAL
PROPERTIES UNDER
PRESSURE

Applicants are invited for the post of Research Associate to work with Dr R G Ross in the School of Mathematics and Physics on the measurement of thermal conductivity and heat capacity of solids and liquids at high pressures and temperatures, using the transient hot-wire method. The post is tenable for three years from October 1985 and has a starting salary of up to £7,980 per annum on the RA (1B) scale (under review). Applicants should have a good honours degree in physics or a related subject, and may register for a higher degree.

A curriculum vitae and the names of two referees should be sent to the Senior Administrative Assistant, School of Mathematics and Physics, University of East Anglia, Norwich NR4 7TJ. (7478)A

UNIVERSITY OF
CAMBRIDGE

University Chemical
 Laboratory

POSTDOCTORAL
RESEARCH ASSISTANT

Applications are invited for two posts of Postdoctoral Research Assistant to work with Dr D Williams on the structure elucidation of antibiotics and peptides. Previous experience with HPLC, proton nuclear magnetic resonance and/or FAB mass spectrometry will be a advantage. Appointments will be made for one year in the first instance, and will commence in the period 1 October 1985 to January 3 1986. Initial salary will be either £7,520 or £7,980 per annum.

Applications, which should include a curriculum vitae together with the names of two referees should be sent to Dr D H Williams University Chemical Laboratory Lensfield Road, Cambridge, CB2 1EW. (7457)A

UNIVERSITY OF
LIVERPOOL
DEPARTMENT OF GEOLOGY
TECHNICIAN GRADE 3

Required in the recently established Sedimentology Laboratory. The successful applicant will be responsible for operating a range of sedimentological research techniques, assisting in the training of students in these techniques and maintaining the laboratory. Experience of grain-size analysis, heavy mineral separation, cathodoluminescence microscopy, impregnation and staining techniques as applied to sediments and sedimentary rocks would be an advantage.

Candidates must possess O.N.C. plus three years experience as a minimum qualification but applications from graduates in Earth Sciences will also be welcomed.

Salary within the range £5,399 — £6,325 pa (under review).

Application forms may be obtained from the Registrar, The University, PO Box 147, Liverpool, L69 3BX.

Ref: PER/754/N (7444)A

MEDICAL RESEARCH
COUNCIL
National Institute for
Medical Research
DIVISION OF
IMMUNOLOGY

Applications are invited for a post-doctoral position in the Division of Immunology to work with Dr B Askonas. The appointment will be for three years, starting in late autumn, to work on aspects of T-cell function and recognition in respiratory virus infection (particularly influenza) in a mouse model. Experience in cellular immunology, tissue culture or virology would be an advantage.

The salary will be in the range of £8,920 — £10,720 per annum, plus £1,233 London allowance and superannuation provision.

Applications, together with curriculum vitae, research experience and interests in the names of two professional referees, should be sent to the Assistant Director, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA by 13 September 1985, quoting ref IMM/1. (7452)A

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 4 Little Essex Street
 London WC2R 3LF
 Tel 01 240 1101
 Telex 262024

TORONTO OFFICE

Peter Drake & Associates
 17 Pine Crescent
 Toronto, Ontario M4E 1L1
 Tel: (416) 690-2423

NEW YORK OFFICE
 65 Bleecker Street
 New York, NY 10012
 Tel: (212) 477-9600

THE BLOOD CENTER OF SOUTHEASTERN WISCONSIN

Immunogenetics
Research Section

POSTDOCTORAL FELLOW

Human Immunogenetics

Immediately available two-year position working in areas of histocompatibility, transplantation and regulation of human T-cell responses to influenza virus, including: graft vs host disease, T-cell clone biology, immunochemistry of T-cell surface molecules, structure and function of human alloantigens.

Send vitae to: David D. Eckels, PhD, Blood Center Southeastern Wisconsin, 1701 West Wisconsin Avenue, Milwaukee, Wisconsin 53233. (NW173)A

NORTH EAST BIOTECHNOLOGY CENTRE

Applications are invited for the following post which will be based at:

TEESSIDE POLYTECHNIC in the DEPARTMENT OF CHEMICAL ENGINEERING PRINCIPAL LECTURER IN BIOCHEMICAL ENGINEERING

To take responsibility for the operation and further development of the Biochemical Engineering content of courses in process engineering run jointly by the Department of Chemical Engineering and the North East Biotechnology Centre.

It is expected that the successful candidate will also undertake research projects, especially in aspects of downstream process design.

Applicants should be well qualified in Biochemical Engineering or a discipline of fundamental importance to this subject area. They should have demonstrated a proven research record in a relevant area, an effective approach to student teaching and appropriate experience. Members of an appropriate professional institution would be an advantage.

Salary: £13,095 — £14,580 (work bar) — £16,467 per annum.

The salary on appointment will be no greater than £14,580 per annum.

Application forms and Further Particulars from Personnel Section, Teesside Polytechnic, Borough Road, Middlesbrough, Cleveland, TS1 3BA, Telephone (0642) 218121 Ext. 4114.

Closing date for applications: 16 September 1985.

An Equal Opportunity Employer (7446)A

UNIVERSITY OF ST ANDREWS

Department of Anatomy
and Experimental Pathology

Applications are invited for a

POSTGRADUATE RESEARCH ASSISTANT 1B

to work with Dr P. E. Bryant on a project, funded by the Cancer Research Campaign, to investigate mechanisms of, and factors affecting the induction of chromosomal aberrations in mammalian cells. The person appointed will use recently developed techniques in which the DNA of living cells is cut with restriction endonucleases. DNA and chromosomal damage arising from this treatment as well as from exposure to X-rays will be investigated using a variety of biochemical and cytogenetical methods.

The post is for 3 years, starting in October, 1985. Starting salary within range £6,600 — £7,055 on 1B scale. Experience with cytogenetical and tissue culture techniques would be an advantage. Further details may be obtained from Dr P. E. Bryant.

Applications, with the names of two referees, should be sent to the Establishments Officer, The University, College Gate, St Andrews, Fife KY16 9AJ by 16th September, 1985 (7404)A

UNIVERSITY OF BRISTOL

Department of Biochemistry

COMPUTER MANAGER

(other related 1A)

Applications are invited for the post of Computer Manager. The duties of the Computer Manager will be to assume responsibility for the operation of molecular graphics equipment and for computing facilities within the Department of Biochemistry. It is also expected that the Computer Manager will take part in research on protein structure and function. Current structurally related research projects in the Department concern glycolytic enzymes, transferrin and site directed mutagenesis.

The appointment is jointly funded by the SERC and the University of Bristol and initially will be for a period of 3 years. Starting salary will be up to £8,450 pa.

Further particulars may be obtained from Dr J. J. Holbrook, Dr H. Muirhead or Dr H. C. Watson, Department of Biochemistry, University of Bristol, BS8 1TD. Applications (3 copies) should include curriculum vitae and names and addresses of 2 referees (7456)A



MICROBIOLOGIST

Plant Protection Division's world research centre is at Jealott's Hill, where we are seeking to strengthen one of our research teams in Agricultural Microbiology by the addition of an innovative research scientist. In particular the new areas of interest will be in developing model systems for the analysis of the Mode-of-Action of pesticides, and isolating bacteria capable of degrading pesticides or resistant to pesticides. There are lots of opportunities for initiative in this exciting area of research. We are looking for a graduate with a first class honours degree or 2(i) in Microbiology and with an M.Sc or experience in biochemistry.

Jealott's Hill Research Station is pleasantly situated between Bracknell and Maidenhead. We offer competitive salaries and other benefits include profit sharing and pension. There is a staff restaurant, recreation club and shop on site.

Please apply with full details of your age, qualifications and experience to: Personnel Department, ICI Plant Protection Division, Jealott's Hill Research Station, Bracknell, Berkshire RG12 6EY. Please quote reference: Microbiol/KAP.

The closing date for this vacancy is 6 September 1985



Plant Protection
Division

(7482)A

RESEARCH ASSISTANT PROFESSOR (non-tenured track)

to study bacterial chemotaxis as part of a research group. Background in molecular genetics or protein structure/chemistry preferred. Five-year position involves light teaching load.

Salary \$24,000 to \$28,000, depending on qualifications. Must have Ph.D and postdoctoral training.

Send curriculum vitae and three reference letters to: Dr. G.L. Hazelbauer, Biochemistry/Biophysics, Washington State University, Pullman, WA., 99164-4660.

Washington State University is an Equal Opportunity/Affirmative Action Employer. (NW164)A

AGROGEN BIOTECHNOLOGIES INC. RESEARCH SCIENTIST — PROJECT LEADER ECOPHYSIOLOGY/ MYCORRHIZAE

Agrogen Biotechnologies Inc. a Vancouver based Canadian company interested in plant tissue culture, micropropagation of woody ornamentals and forest tree species and related biotechnologies is seeking a Research Scientist to act as Leader of its research project on ecophysiology of mycorrhizae symbiosis.

The position requires a Ph.D. in Forestry with specialization in ecophysiology and ectomycorrhizae, post-doctoral experience in water relations and ecophysiological evaluation of plant growth responses, demonstration of supervisory skills through the conduct of complex research projects and demonstration of independent scholarly research through publication in recognized scientific journals.

Agrogen offers the opportunity to join an energetic interdisciplinary research team, excellent research facilities competitive salary and fringe benefits.

Please forward your curriculum vitae and other pertinent information including the name of 3 references by September 30, 1985, to: Dr. D. Paul Webb, Director of Research, Agrogen Biotechnologies Inc., 1710 — 1066 West Hastings St., Vancouver, B.C., V6E 3X2, Telephone (604) 683-0345. (NW172)A

Experimental Scientist (X-Ray Diffraction) Synchrotron Radiation Research

The Daresbury Laboratory, one of the Science and Engineering Research Council's major laboratories, situated in the North Cheshire countryside, is developing large scale national facilities for research into atomic, molecular and biological sciences, nuclear physics and computational science. In particular, the Synchrotron Radiation Source (SRS) supplies X-radiation, UV and IR to over 20 experimental stations where new and exciting research programmes are being carried out.

We have a vacancy in the Synchrotron Radiation Division for a person who would be developing facilities for powder diffraction and single crystal work, including surface diffraction. A two-circle instrument is already installed in the intense X-ray beam from the SRS wiggler magnet and a four-circle instrument is being purchased. We need a person with a strong background in physics, chemistry or materials science with practical experience in the application of diffraction techniques. The person appointed will assist a wide range of instrument users in the development of their research programmes and play a leading role in the application of the two-circle and four-circle instruments to new scientific problems. Dedication, enthusiasm and a willingness to form part of a team of hard working scientists are all essential pre-requisites for this post.

Depending on qualifications and experience the appointment will be made in the grade of Scientific Officer or Higher Scientific Officer on a salary scale of £6,190 to £8,561 or £7,788 to £10,541 per annum. There is a non-contributory superannuation scheme, a generous leave allowance and a flexible working hours scheme. Applicants should have a degree or equivalent in a relevant subject.

For further information please contact the Personnel Officer, from whom application forms may be obtained and should be returned quoting reference number DL/927 to:

The Personnel Officer, Daresbury Laboratory,
Science and Engineering Research Council,
Daresbury, Warrington,
Cheshire WA4 4AD.
Tel Warrington (0925)
65000, Ext. 467

CLOSING DATE: 13th Sept 1985
(7489)A

Daresbury
SCIENCE & ENGINEERING
RESEARCH COUNCIL

LA TROBE UNIVERSITY Melbourne, Australia

School of Biological Sciences RESEARCH ASSOCIATE IN BIOCHEMISTRY

A Research Associate is required to work on cyclic AMP metabolism and Ca^{2+} mediated processes (including protein phosphorylation) in plants. The appointee will be a member of a research group headed by Dr Gideon Polya in the Department of Biochemistry. It is expected that the successful applicant will commence in 1985. The position is funded by the Australian Research Grants Scheme for a period of 2 years. Applicants must have a PhD in biochemistry or a closely related field. Further details of the position may be obtained from Dr Polya by writing or telephoning (03) 478 3122 Ext 2157. Applicants should ask two referees to send their confidential reports to the Staff Officer, La Trobe University, by the closing date, or as soon as possible thereafter.

Salary: \$A22,614 — \$A23,934.

Applications marked 'Confidential' and quoting AO/004/002, close with the Staff Officer, La Trobe University, Bundoora, Victoria, Australia 3083, on 16 September 1985. Candidates in UK should also send a copy of their applications to the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, from whom further general information is available.
(W2148)A

MEDICAL RESEARCH COUNCIL CLINICAL & POPULATION CYTOGENETICS UNIT BIOLOGIST

Applications are invited for a Short-Term Non-Clinical Scientific post in the above Unit, tenable for 3 years from October 1985. Candidates of immediately post-doctoral or equivalent status, and having some experience in cytogenetics or relevant molecular biology will be preferred. The appointee will work with a group using DNA probes in studies on chromosome anomalies in normal and malignant human cells.

Remuneration will be at an appropriate point on the scales for University non-Clinical academic staff. Further details from, and applications, with names and addresses of two referees, to the Administrator, MRC Clinical and Population Cytogenetics Unit, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, by September 9th, 1985, quoting reference HJ/8/2989
(7462)A

Please mention
nature
when replying to
these advertisements

FACULTY POSITION: RHEUMATOLOGY AND IMMUNOLOGY

Research position for MD to conduct an independent research program in Rheumatology-Immunology with pertinence to the rheumatic diseases (rheumatoid arthritis).

Will supervise research projects of trainees in rheumatology, and will participate in teaching programs in rheumatology and immunology. Will receive academic appointment commensurate with his/her qualifications.

Submit curriculum vitae and names of three references to: D L Conn, MD, Division of Rheumatology, Mayo Clinic, Rochester, MN 55905.

An Equal Opportunity / Affirmative Action institution.

(NW160)A

UNIVERSITY OF LONDON Imperial College of Science and Technology POSTDOCTORAL RESEARCH ASSISTANT

required for a 2 year appointment to do in-line measurement and computer analysis of electro-cardiographic signals and their interpretation in relation to a computer model of the human thorax, in collaboration with St Mary's Hospital.

The successful candidate will be expected to have a postgraduate degree in Electrical Engineering and Computer Science, a knowledge of real-time programming and experience in physiological measurement in a clinical environment. Salary will be on a scale £8,753 — £13,383 (under review).

Applications to Dr D M Monro, Department of Computing, Imperial College, SW7 2AZ by 12 September 1985
(7486)A

UNIVERSITY OF LONDON Institute of Obstetrics and Gynaecology — Hammersmith Hospital, Ducane Road, London W12 0HS POST DOCTORAL RESEARCH ASSISTANT

required to study the hormonal regulation of protein synthesis in human endometrial carcinoma. The project will involve tissue culture and analysis by PAGE of cellular and secreted proteins labelled in-vitro. Previous experience of these techniques is desirable but not essential. The position is available for 3 years and is funded by a project grant from the Cancer Research Campaign. Salary up to £11,205 inc of LW.

Applications in the form of a CV including the names of 2 academic referees should be sent to Dr J O White at the above address. Informal enquiries can be made by telephoning (01-743-2030 Ext 547)

Closing date 19 September 1985.
(7485)A

UNIVERSITY OF LEEDS DEPARTMENT OF PHYSICS POSTDOCTORAL RESEARCH FELLOW

(Astrophysics)

Applications are invited for the above post for work with Dr A Hillas on modelling the emission ultra high energy (10000 TeV) radiations from Cygnus x-3 and related objects, as a probe of the environment of close binary stars, and gain insight into the origin of cosmic rays.

Applicants should have a PhD or relevant research experience. The post, funded by the SERC, is available for a fixed term of up to two years.

Salary within the range £7,520 - £8,920 (under review) on the 1. Range for Research and Analogous Staff according to age, qualification and experience.

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference number 52/33, closing date for application 12 September 1985. (7442)A

UNIVERSITY OF CAMBRIDGE POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for a postdoctoral research assistant to work on the processing of transcripts of chloroplast DNA from higher plants. This will include the removal of introns from transcripts of protein-coding genes. Experience of molecular biology techniques would be an advantage. The position which is supported by SERC, is for a period of three years from 1 October 1985, or as soon as possible thereafter. The salary will be on the National 1A scale.

Applications with curriculum vitae and the names and addresses of two referees should be sent as soon as possible to Dr J C Gray, Botany School, University of Cambridge, Cambridge CB2 3EA, from whom further details may be obtained
(7483)A

ASSISTANT EDITOR

Leading German scientific/technical publisher seeks young science graduate (preferably chemistry, physics) with excellent command of English (native speaker) for editorial duties (initially copy editing) related to English-language subscription journals in the area of analytical chemistry, analytical instrumentation, etc. Previous publishing experience highly desirable, knowledge of German advantageous. Location will be in historic city in South-West Germany. This vacancy is to be filled in the near future.

Applications with CV and photo to Box Number W2131, c/o Nature, 4 Little Essex Street, London WC2R 3LF.
(W2131)A

FELLOWSHIPS

**ST GEORGE'S HOSPITAL
MEDICAL SCHOOL**
(University of London)
**POSTDOCTORAL
RESEARCH FELLOW**

One year Fellowship available from 1st November 1985 in a sponsored (Glaxo) research project involving characterisation and DNA mapping of proteins that regulate chromatin structure in mammalian cells.

biochemistry background essential, demonstrated expertise in molecular biology or DNA enzymology offered. Initial salary in range £520—£8,920 plus London Allowance £1,233 pa.

For further details contact Dr L M Sher, Biochemistry Department 1-672-1255 ext 4760. Application form from Personnel Officer, St George's Hospital Medical School, Cranmer Terrace, London SW17 3E. Tel 01-672-1255 ext 4873. Closing date 16th September. Quote 11/ (7475)E



UNIVERSITY OF DUBLIN
Trinity College

DEPARTMENT OF GENETICS

Applications are invited for a Research Fellowship in the field of

**BIOINFORMATICS/
BIOCOMPUTING**

The position is available for one year in the first instance.

The project concerns the establishment of a national centre for DNA and protein sequence analysis. Activities will include design and coordination of appropriate software for use by and for molecular geneticists, biochemists and microbiologists.

Applicants should have experience in computing, and an interest in molecular biology. Salary, depending on experience, up to IR£10,268 pa.

Applications should be submitted to Dr. P. Smith-Keary, Department of Genetics, Trinity College, Dublin 2 by 16th September 1985.

(7484)E

JOINT IMER/WRC POSTDOCTORAL FELLOWSHIP

Modelling Population Processes

The Institute for Marine Environmental Research and the Water Research Centre are offering a 2 year research fellowship to develop a computer simulation model relating physiological traits in marine invertebrates to features of population dynamics. Candidates should have completed a Ph.D. in the biological sciences and have experience in computer modelling.

Appointment will be at a grade equivalent to Higher Scientific Officer, with starting salary according to age, qualifications and experience in the range £7,788 to £10,541. Conditions of service are similar to those of the Civil Service.

For further information and applications form write, giving brief details of qualifications and experience to The Director, NERC, Institute for Marine Environmental Research, Prospect Place, The Hoe, Plymouth PL1 3DH, quoting Post No: A3.

Closing date: 30 August 1985.

(7465)E



Natural
Environment
Research
Council

GRANTS & SCHOLARSHIPS

**UNIVERSITY OF
NOTTINGHAM**

**Department of Applied
Biochemistry & Food Science**
**MAFF POSTGRADUATE
SCHOLARSHIP**

**Development of Methodology
for Meat Identification in Foods**

Following demonstration that the concentration of 3-methyl-L-histidine provides a robust, unequivocal index of the proportion of animal protein in foods which have been too severely processed to permit the use of electrophoretic or immunological techniques, the methodology has been refined and developed. Further investigation of this, and related procedures in food analysis is being supported by a Postgraduate Scholarship over the 3-year period 1985-88.

Remuneration (and appropriate allowances) will be at the rates advertised in "Postgraduate agricultural scholarships, 1985," Ministry of Agriculture, Fisheries & Food, London.

Applications are invited from those holding a 1st or upper 2nd class honours degree in Food Science, Chemistry or Biochemistry. Experience in column chromatography (preferably in high performance liquid chromatography) would be an advantage.

The successful applicant will be expected to read for the PhD degree of the University of Nottingham under the supervision of Professor R A Lawrie.

Applicants should send a cv (including the names of 2 academic referees) to the Secretary, Faculty of Agricultural Science, University of Nottingham, Sutton Bonington, Nr Loughborough, LE12 5RD (7470)H

WRC/UWIST John L van der Post Fellowship in Biotechnology. Stability of cloned catabolic genes and recombinant host bacteria in activated sludge. Applications are invited for the above Fellowship which is funded for three years by the Water Research Centre (WRC) to start from January 1 1986. Applicants should have a PhD, or expect to obtain one by the starting date, in microbiology, genetics or a related discipline. Experience of recombinant DNA techniques would be a definite advantage. The research, which will be carried out at both UWIST and WRC Processes laboratory in Stevenage will be supervised primarily by Dr J C Fry and Dr A J Weightman of UWIST. Advice will also be given by other UWIST and WRC staff when required. The project will involve the application of techniques for genetic manipulation to the analysis of heterogeneous microbial populations into which defined genetic markers will be introduced. The effects of manipulations on the stability of cloned genes encoding enzymes of halogenated aromatic catabolic pathways will be examined. Survival of recombinant host bacteria inoculated into laboratory and larger scale activated sludge plants and cloned gene transfer in the mixed microbial culture will be analysed by DNA hybridization and fingerprinting techniques. The Fellowship will be on the research and analogous staff scale 1A and will start in the range £7,520—£12,150 according to age. Applications in the form of a letter of application, a CV and the names of two referees should be sent to: Dr J C Fry, Department of Applied Biology, UWIST, PO Box 13, Cardiff CF1 3XF. Further details can also be obtained from the above address if required. The closing date for applications is 13 September 1985 (7476)F

ASSISTANTSHIPS

UMIST

**DEPARTMENT OF
MATHEMATICS**

**RESEARCH
ASSISTANTSHIP**

A Research Assistant is required for one year from 1 October 1985 to work in the field of integrable system and solutions. Salary (under review) up to £9,390 pa depending on age and experience.

Further information available from Professor R K Bullough, Department of Mathematics, UMIST PO Box 88, Manchester M60 1QD, UK. (Tel UK 061-236 3311 ext 2189) to whom curriculum vitae, quoting reference MAT/132, and list of publications should be sent (7477)P

UNIVERSITY OF BRISTOL

School of Chemistry
ORGANIC GEOCHEMISTRY UNIT
Chemical Fossils

**RESEARCH
ASSISTANTSHIP**

(Grade 1B, NERC funded) for three years, to investigate organic compounds in oceanic sediments. Applicants (under 27) should possess an honours degree in chemistry, geochemistry or biochemistry and be analytically adept and computationally competent.

Applications (enclosing CV and referees' names) to: Dr S C Brassell, Organic Geochemistry Unit, University of Bristol, School of Chemistry, Cantock's Close, Bristol, BS8 1TS (7470)P

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WORKSHOPS

THE OCEAN DRILLING PROGRAM calls for the drilling vessel JOIDES RESOLUTION to operate in the Pacific Ocean during all or parts of 1989-1990

In order to develop a drilling program that will address problems in tectonics, the nature of the lithosphere, paleoceanography and sedimentology processes in the South Pacific and the Arctic margin, a planning workshop will be held at the University of Florida (Gainesville) during mid to late April 1986. The workshop is funded by the Joint Oceanographic Institutions. Financial assistance is available to US scientists through the workshop organizers. Potential participants should submit by December 1 1985 a summary of contributions they could make to the meeting.

Submit correspondence to any of the following meeting organizers: P. Ciesielski, Department of Geology, University of Florida, Gainesville, FL 32611; J. Mammerrickx, Scripps Institution of Oceanography, La Jolla, CA 92093; J. Weissel, Lamont-Doherty Geological Observatory, Palisades, NY 10964; J. Anderson, Department of Geology, Rice University, Houston, TX 77251.

(NW168)V

STUDENTSHIPS

THE ANIMAL VIRUS RESEARCH INSTITUTE RESEARCH STUDENTSHIP

Applications are invited from recent graduates with a 1st or upper 2nd class honours science degree in medicine or veterinary medicine for a research studentship leading to the PhD degree.

It will be in the Division of Immunology and Pathology, for studies of the role of cell-mediated immunity in African swine fever infection.

Apply as soon as possible, enclosing a CV and the names of two referees to the Secretary, The Animal Virus Research Institute, Ash Road, Pirbright, Woking, Surrey GU24 0NF.

(7387)F

THE UNIVERSITY OF WARWICK POSTGRADUATE STUDENTSHIP

In The Department
of Chemistry

Applications are invited for an industrially sponsored studentship (funded at SERC rate) to study electrochemical methods of enzyme immobilisation. The project will involve the development of electrochemical immobilisation techniques, the characterisation of the immobilised enzymes, and their application in chemical sensors.

Candidates should have a first or upper second class honours degree, or equivalent, in Chemistry or a related subject.

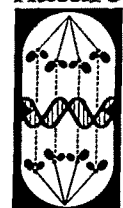
Applications, together with the names of two referees, should be sent as soon as possible to The Registrar, University of Warwick, Coventry CV4 7AL, quoting Ref No 2/2A/85/0. Further details may be obtained from Dr P N Bartlett (Tel 0203 24011 Ext 2264).

(7460)F

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UNIVERSITY OF BRISTOL

Long Ashton
Research Station
**RESEARCH
STUDENTSHIP**

Applications are invited from candidates with a good honours degree in Plant Sciences for a 3-year project leading to a PhD, commencing in October, 1985. The student will join a small team funded by the Overseas Development Administration to investigate methods of controlling the tropical perennial grass, *Imperata cylindrica*, by methods appropriate to small-scale farmers.

Research for the PhD will be based on herbicide application by weed wipers and will involve studies on the placement, uptake and translocation of concentrated herbicide solutions using techniques which will include isotopes and fluorimetry.

A curriculum vitae and the names and addresses of two referees should be sent to The Administrator, Weed Research Division, Long Ashton Research Station, Begbroke Hill, Yarnton, Oxford, OX5 1PF by 5th September, 1985. Further information is available from Mr P J Terry who will be pleased to receive any telephone enquiries (08675 3761).

(7464)F

UNIVERSITY OF MANCHESTER

Department of Surgery
RESEARCH STUDENTS

Applications are invited for a studentship (funded by Janssen Pharmaceuticals Ltd) in collaboration with the MRC Trauma Unit to study changes in regional cerebral blood flow after injury and how these changes are modified by treatment.

Applicants should have a first upper second class honours degree in physiology, pharmacology or a related subject. The successful applicant will be expected to register for a higher degree.

Applications including CV and names of two referees should be sent as soon as possible to Professor Irving, Department of Surgery, Hope Hospital, Eccles Old Road, Salford M6 8HD. (7480)

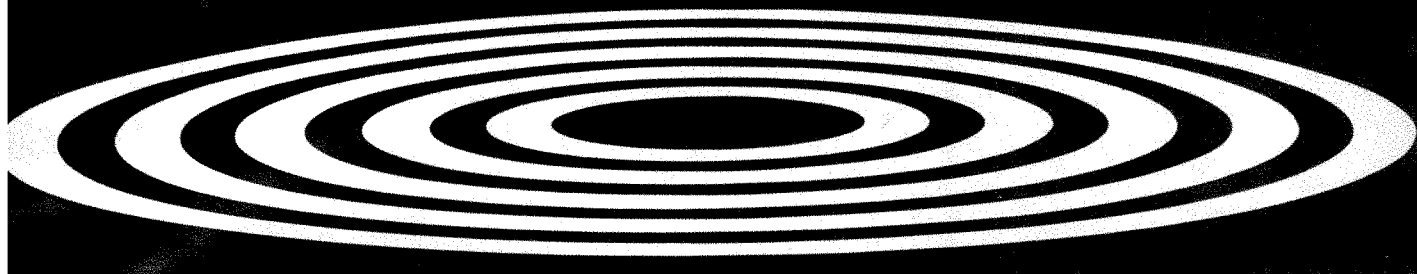
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The speakers from the United States will include Nobel Prize winners **James Watson** (*Cold Spring Harbor*), who (with F.H.C. Crick) first suggested the structure of DNA, and **Walter Gilbert** (*Harvard University*). Other speakers include: **Stanley Cohen** (*Stanford University*), **Tom Blundell** (*Birkbeck College, London*)

Walter Gehring (*University of Basel*)

Richard Lerner (*Scripps Clinic, La Jolla*)

Sydney Brenner (*Laboratory of Molecular Biology, Cambridge*)

Susumu Tonegawa (*Massachusetts Institute of Technology*)

Shosaku Numa (*Kyoto University*)

Tasuku Honjo (*Kyoto University*)

Ken-ichi Matsubara (*Osaka University*)

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The three main topics to be covered by the conference are

- Molecular biotechnology
- Development and regulation
- Immunology and virology

and the programme is being designed to provide an opportunity for those working in industry, medicine or academic research to bring themselves up to the forefront of research through discussion with world leaders. There will be ample time for questions and discussion. Simultaneous translation services into English and Japanese will be available.

Registration fees:
¥80,000 (before 30 November, 1985)
¥100,000 (after 1 December, 1985)

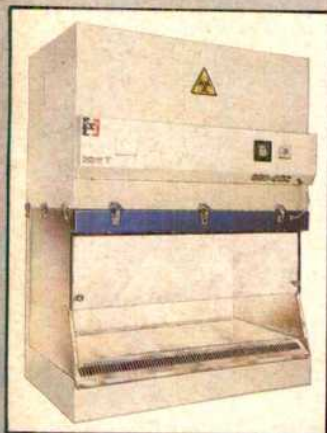
Full details are available from:

Nature Conference Secretariat International Conference Organizers, Inc.
103, Crescent Plaza
4-6, Minami-Aoyama 2-chome
Minato-ku
Tokyo 107
Japan
Tel: 03-470-3766
Fax: 03-405-8638
Cable: CONFERENCEORGAN
TOKYO

A limited number of tickets for young people will be available at ¥20,000 each. Those under the age of 35, who are carrying out research in molecular biology or a related field and do not have access to funds to pay the full registration fee, should write to the Nature Conference Secretariat for an application form.

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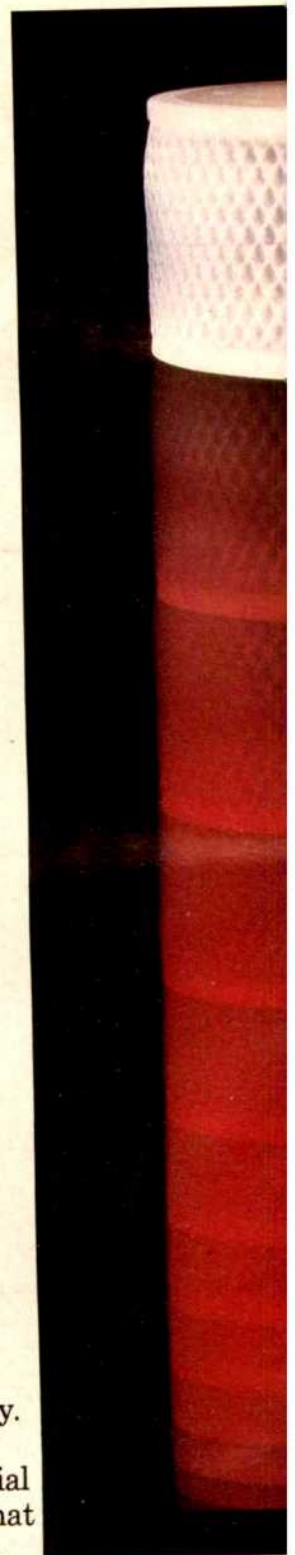
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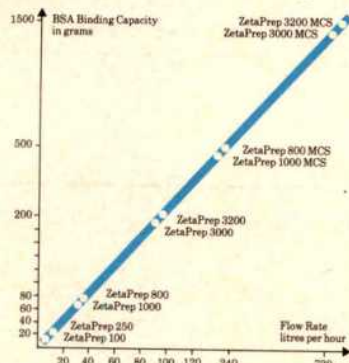
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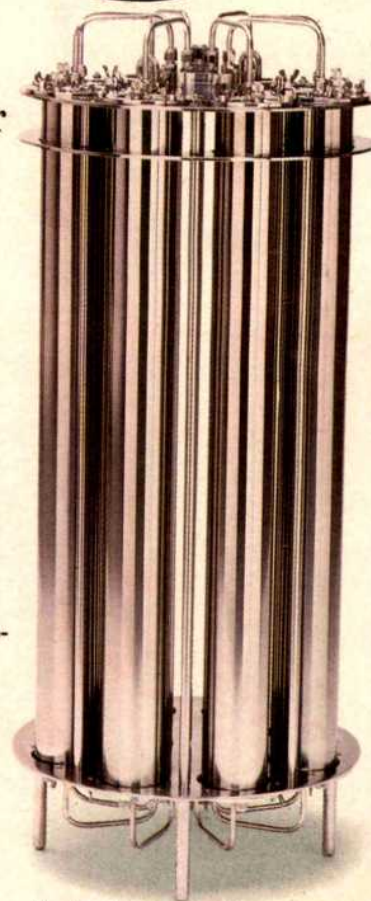
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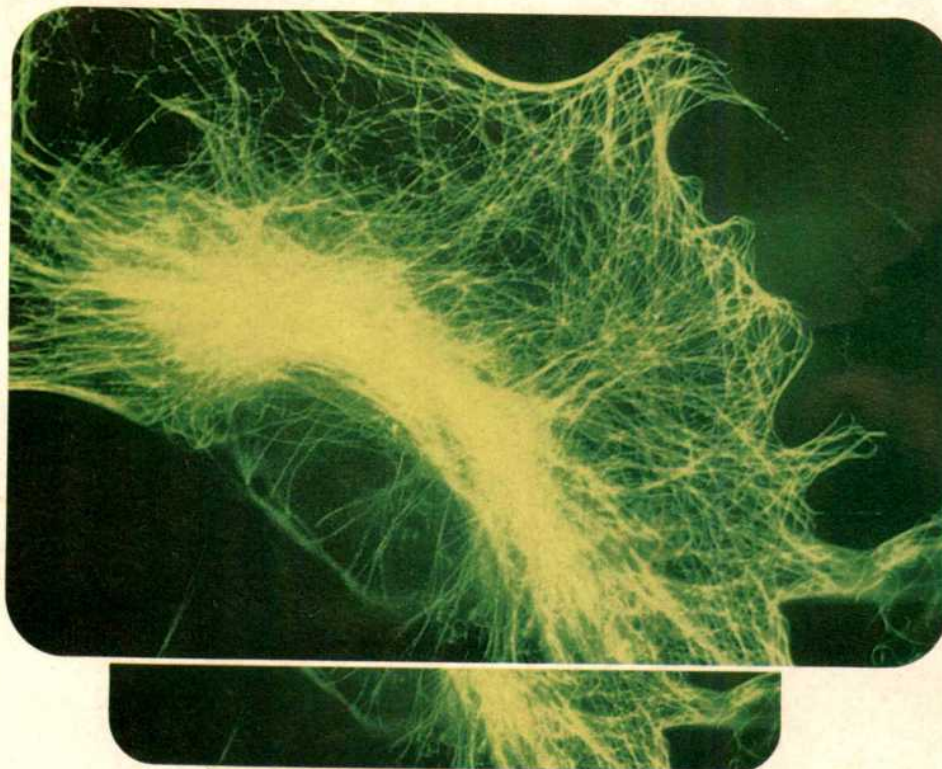
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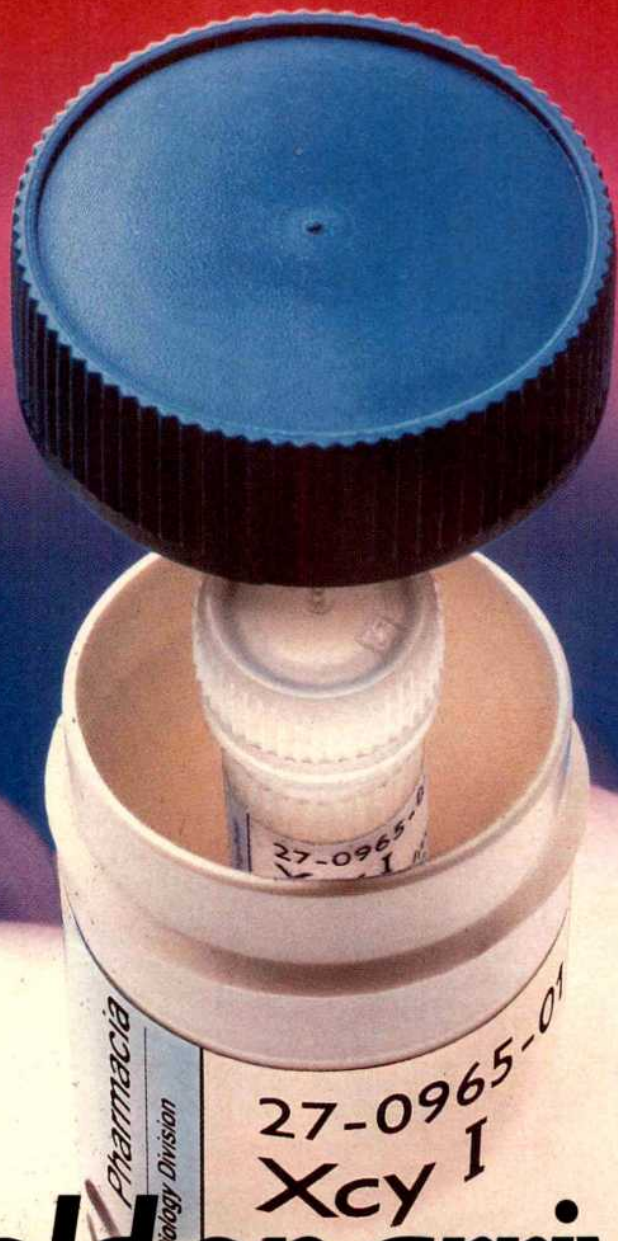
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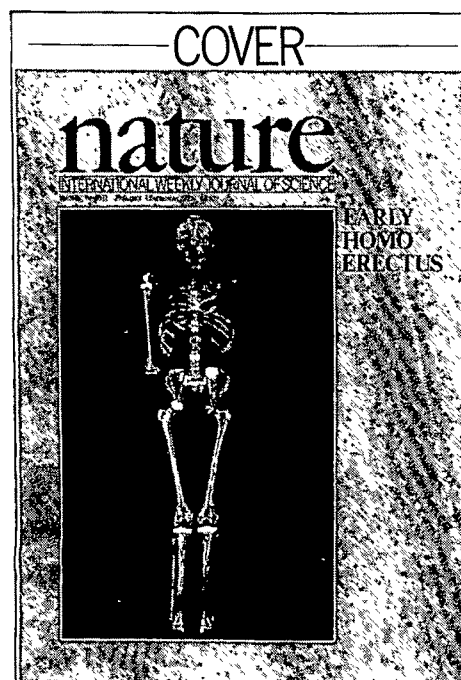
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A coloured replica of *Homo erectus* skeleton KNM-WT 15000, the most complete early hominid skeleton yet found. Discovered near Lake Turkana in Kenya, it was excavated from sediments dated at 1,600 million years old. Colour code: fawn, parts modelled in mirror-image; yellow, parts added for mounting purposes only, green, intervertebral disks, epiphyseal plates and filler on the cranium for casting purposes. See page 788.

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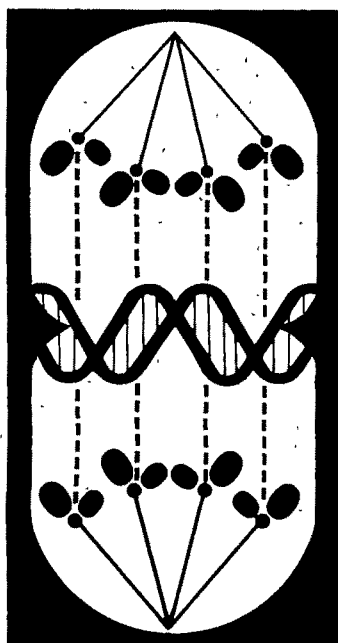
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DAY 1 MONDAY, 7 OCTOBER

8 45-8 50	Chairman	
8 50-9 40	<i>Drosophila</i> early development	<i>C. Nüsslein-Volhard, Max Planck, Tübingen</i>
9 40-10 30	Genetic control of <i>Drosophila</i> development	<i>D. Hogness, Stanford University</i>
10 30-11 10	COFFEE	
11 10-12.00	Comparative expression of homeobox genes in fly and mouse	<i>W. McGinnis, Yale University</i>
12 00-1 30	LUNCH	
1 30	Chairman	
1 30-2 15	Cytoplasmic localizations in the amphibian egg	<i>J. Gerhart, University of California, Berkeley</i>
2 15-3 00	Genes that control nematode cell lineage	<i>H.R. Horvitz, Massachusetts Institute of Technology</i>
3 00-4 00	ROUND TABLE with day's speakers	
4 00-4 30	TEA	
4 30-5 30	Closing talk	<i>G. Stent, University of California, Berkeley</i>

DAY 2 TUESDAY, 8 OCTOBER

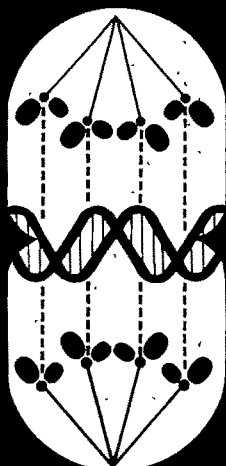
8 45-8 50	Chairman	
8 50-9 40	A master regulatory locus that determines cell specialization in yeast	<i>I. Herskowitz, University of California, San Francisco</i>
9 40-10 30	Regulatory proteins in bacteria and yeast	<i>M. Ptashne, Harvard University</i>
10 30-11 10	COFFEE	

11 10-12 00	Simple and combinatorial regulation of gene transcription	<i>K. Yamamoto, University of California, San Francisco</i>
12 00-1 30	LUNCH	
1 30	Chairman	
1 30-2 15	Control of animal growth by gene transfer	<i>R. Palmiter, University of Washington, Seattle</i>
2 15-3 00	Models for human gene therapy	<i>T. Friedmann, University of California, San Diego</i>
3 00-4 00	ROUND TABLE with day's speakers	
4 00-4 30	TEA	
4 30-5 30	Closing talk	<i>E. Jaworski, Monsanto Company</i>

DAY 3 WEDNESDAY, 9 OCTOBER

8 45-8 50	Chairman	
8 50-9 40	Structure analysis of T-cell recognition	<i>M. Davis, Stanford University</i>
9 40-10 30	Expression of immunoglobulin genes in transgenic mice	<i>U. Storb, University of Washington, Seattle</i>
10 30-11 10	COFFEE	
11 10-12 00	Proto-oncogenes: the good and the bad	<i>J.M. Bishop, University of California, San Francisco</i>
12 00-1 30	LUNCH	
1 30	Chairman	
1 30-2 15	Cell recognition during neuronal development	<i>C. Goodman, Stanford University</i>
2 15-3 00	Molecular insights into mammalian systems	<i>F. Bloom, Research Institute of Scripps Clinic</i>
3 00-4 00	ROUND TABLE with day's speakers	
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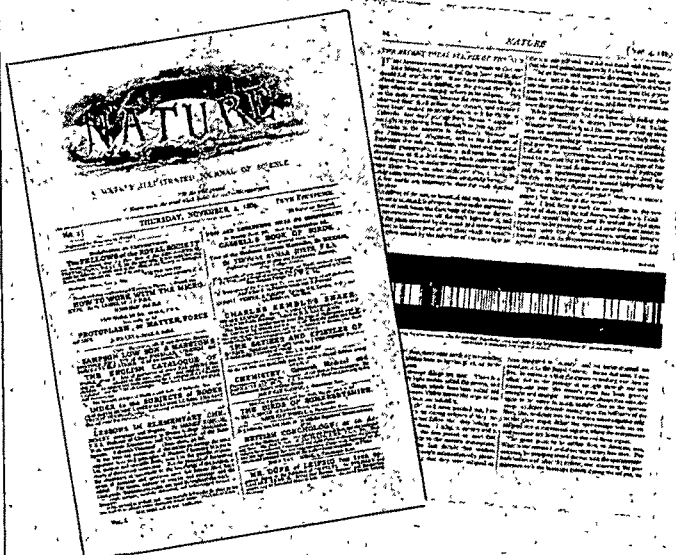
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Spying is getting out of hand

State Department allegations of Soviet surveillance in Moscow will cause much more trouble than the technique can have been worth. The time has come to agree a code of conduct for spies.

JUST as science fiction has a tendency to become fact, the literary genre of the spy thriller is turning into a curious kind of reality. So much is clear from the US State Department's claim last week that the members of its embassy's staff in Moscow have been contaminated with a chemical (mtrophenylpentadiene) which can be made to be fluorescent by irradiation in the ultraviolet. The allegation, on which further details are promised, is that the material has been planted in people's clothing and on, say, the steering wheels of motor cars in such a way that drivers' hands are likely to be contaminated. The announcement lends credence to the idea that, by means like this, it would be possible for Soviet security authorities to learn what members of the US embassy are about during the course of their working day.

There are several things to say, of which the first is that such a practice would be a serious infringement of the rules that should govern the conduct of diplomatic missions, laid down by the Vienna conventions on the subject. The principle is (or should be) that even people who work as diplomats in foreign countries are, like their hosts, entitled to a measure of personal privacy and to the presumption that they are not criminals — unless evidence to the contrary comes to light. That is why the agreements governing the conduct of embassies usually allow for people's freedom to travel within a certain well-defined area, and entail the assumption that meetings with local residents are by definition innocent unless discovered to be otherwise. A little reflection will show that the whole business of international diplomacy would grind quickly to a halt if these assumptions were not valid. Seeking to provide continuing surveillance of what foreign diplomats are up to, as the Soviet Union may have been doing, would be a serious breach of these important principles.

But even if the allegations are borne out by further evidence, what in principle will have been accomplished? The use of chemicals fluorescent in the ultraviolet has a minor place in forensic science as a means of marking objects whose origin is known, but a more conspicuous place in the luxuriant detective-story literature of the West. The obvious difficulty, in the scheme the Soviet authorities are said to have used to track the movements of members of the US embassy in Moscow, is that there is no obvious way in which generalized use of the fluorescent chemical could possibly have been of value to them. It is unthinkable that the authorities could have scoured Moscow with ultraviolet lamps, looking for traces of US diplomats whose clothing had been doped with the tracking chemical. What would be possible is to tell whether some otherwise unknown person seen seated in some public place had been a US diplomat, or whether a particular high-denomination currency bill (a dollar or a rouble) had been peeled off a stack of notes by a person carrying the tell-tale chemical on his fingers. In other words, if such a chemical has been used and has been found

useful by Soviet counter-intelligence authorities, it is more likely that its function has been to help tell what Soviet citizens have been doing than to keep a permanent watch on the movements of US diplomats. But the chances are that a technique like this would have been of only marginal value in the legitimate prosecution of counter-intelligence by the Soviet authorities. If real, the allegations probably point to some over-zealous and impracticable scheme suggested by an over-enthusiastic committee.

Even if diplomats elsewhere are unlikely to be exposed to hazards of this kind, because other police authorities are unlikely to copy the bizarre technique now described, there is not much else that is cheerful at this stage. If the details of the allegation are borne out, they are bound to sour the climate in which the Soviet Union and the United States approach the next few critical months of conversation about arms control, and about their general relationship with each other. That is the opposite of good news. In the United States, thrown into anxiety about national security by recent cases of espionage and by fears that foreign nationals, diplomats and others increasingly function as espionage agents, the result of the disclosures from the Moscow embassy may be to amplify still further the demand for extra restrictions on the movement of people away from the places to which they are formally posted. And the result of that would be a further twist in the depressing game of tit-for-tat that has characterized the relationship between the Soviet Union and the United States for too long now.

So, in a sense, the best hope is that the allegations now made by the State Department turn out to be untrue, or that while the details may be verified, the implication that chemicals have been used as a means of keeping track of diplomats may be mistaken. Unhappily, that is unlikely to be the case. But there is a chance that the Soviet leadership will be able to argue, with reasons given, that any such practices were carried out without its knowledge, perhaps by junior people in some over-eager part of its establishment. Even that would be a great deal to ask for; the Soviet Union has never been happy to confess to imperfections in its way of running its own affairs. Yet this, it seems probable, will be the only escape the government of France will have from the allegations in New Zealand that French agents were somehow involved with the sinking of the Greenpeace vessel at the end of June.

Espionage and its elaborations (sinking ships, assassinating people) may seem to governments necessary to their domestic security but they are also a threat to international security. Is it entirely unthinkable that they should be regulated by international treaty? The obvious difficulty is that all governments engage in espionage but insist that their hands are clean. That is increasingly a sick joke. Moreover, there are at least some circumstances in which the reality of espionage is acknowledged, as when governments trade groups of captured agents at the Berlin Wall or elsewhere. Why not go the obvious step further, and negotiate a kind of code of conduct for intelligence services. An agreement not to take direct action against third parties (such as Greenpeace) would not, after all, constitute an admission that other less foolish practices are matters of routine. President Reagan and Mr Gorbachev might usefully spend a few minutes on the topic at their meeting in November. □

Biological manuscripts

Contributors are reminded that, with the transfer of the Biological Sciences Editor to the Washington office, they should in future send four copies of all manuscripts offered for publication either (as at present) to London or to Washington.

Upsetting apple-carts

The British research establishment is ripe for a shake-up. Here is one recipe.

THE past year has been a bigger trauma for the British research community than even its immediate predecessors, dominated as they were by the dwindling value of the funds available for research. Now, perhaps not before time, structural change is in the air. Several unrelated issues have come to the boil at the same time. The Science and Engineering Research Council, with a new chairman and a substantially changed membership (see p. 756) must soon produce a plan for the years ahead that will give the largest of the research councils a coherence that will hold it together in the face of centripetal tendencies born of its complexity. The University Grants Committee is committed to a plan for concentrating financial support on the universities strongest in research, but neither the committee nor its dependants know how the system will work. The Advisory Board for the Research Councils (ABRC) is busily putting out fires where it can find them, and is for example embarked on an inquiry into the plight of the British Geological Survey, the largest single component of the Natural Environment Research Council (NERC), but seems not yet to have attempted the more urgently needed inquiry into its own constitution.

The immediate effect is that people in laboratories have been painfully reminded of the fate that has already befallen some thousands of researchers and academics, whose jobs have melted away in the past few years. But as the whole world knows, there is nothing so demoralizing as uncertainty. It is in everybody's interest that the changes now under way should be carried through quickly. Things have reached the point at which almost any decisions would be better than the continuing indecision that seems to grip the establishment. There is nothing new in the recipe for change that follows, most of which has been advocated in the past year or more. Other recipes might serve the same purpose. The need is that somebody should pick one of them and stick to it.

Money. The government has kept its promise of five years ago that research council budgets would stay constant, and there has even been some extra money for earmarked purposes such as information technology. It is intolerable that there should have been so little substantial help with the reorganization of laboratories forced on the Agricultural and Natural Environmental Research Councils in the past few years, for the government has thus allowed what it considers to have been past mistakes to cast a shadow over the health of the whole enterprise. But people had better learn to live with the notion that there will be no extra funds. But reorganization is necessary for other reasons than financial shortage.

Management. The touchstone for the past dozen years in the management of British civil science has been the Rothschild principle, by which government departments, proxy "customers" representing the interests of the end users of research, commission much of the work carried out in civil research establishments. That principle is now dead, as shown by the ease with which government departments have been able to walk away from research institutes they had previously supported. (The British Geological Survey is that most conspicuously left high and dry by departmental fickleness of this kind.) So there is a need for other mechanisms for supporting important and creative work. To detach some parts of the publicly supported research network altogether from the government would make sense. ("To privatize" is the fashionable verb.) The Plant Breeding Institute, perhaps the most immediately productive of the agricultural research council's establishments, is the obvious candidate if it were allowed also to enjoy the income generated by its findings through the National Seed Development Organization; why does nothing happen? But if the government hopes that the cost of public research could be drastically reduced by hiving off large chunks of it, that ambition should be suppressed. Nor is there as much hope as there may seem in

schemes for encouraging parts of the research enterprise to compete for research contracts with private industry; the universities and private research organizations are now eagerly exploiting that market, the former at cut-price rates. Deciding what parts of the public research network to keep and to cherish, and what parts to close down, is an urgent need. Since many of the neglectful government departments are probably mistaken in their indifference to research, the British government corporately should take a view on the wisdom of transferring organizations such as the institutes responsible for geological survey and oceanography back to where they came from (the Department of the Environment and the Royal Navy, respectively).

Leadership. One of the contributory causes of the present decline of British science is that working scientists no longer have a sense that there is a powerful and influential group of people looking out for the well-being of their work. The heads of the research councils are mostly reasonable men, but are undemonstrative and, by tradition, are also anxious not to give the impression publicly that they seriously dissent from the way in which science is being managed, or mismanaged. The result is that they seem to their constituents more like civil servants than the spokesmen of their subcommittees. In the peculiarly British environment, the Royal Society might most suitably play this role. But would the task of helping to point the scientific enterprise in the right direction be feasible within the convention of politeness to which the society is committed?

Institution. Many of the organizations responsible for the direction of civil research in Britain have outlived their usefulness, or have reached the point where only substantial change will fit them for what emerges as their purpose. ABRC itself is a point at which reform should start. The council is too large to be a workable committee, and too representative of conflicting interests to be an effective way of settling contentious issues. To the extent that the council's function is to advise the Secretary of State for Education and Science, one man would suffice. To the extent that it is necessary to put forward the opinions of the users of what the research councils provide — that there should be some predetermined pattern of spending — a handful of outsiders would be the ideal. To the extent that the council should have a part to play in helping to understand what is happening to British research, there is a need for a more effective secretariat than at present. All this is roughly what Sir Douglas Hague, the chairman of the Economic and Social Research Council and one of the members of ABRC, was saying when he joined the council two years ago. Why have his fellow members not listened to what he was saying? And why have the same people not understood the case for disbanding NERC, the research council that had the bad luck to be set up with terms of reference too ambitious for the components with which it was endowed.

Coordination. One of the chief diseconomies in the present organization of research in Britain is that there is no way in which the different parts of the research enterprise can be meshed with each other. Defence research is an entity on its own (but change may be on the way). And separate government departments continue to maintain laboratories and research institutes which become, as morale sinks deeper, more separate from each other than they have ever been. The National Physical Laboratory, dependent on the Department of Trade and Industry, has been in its time a powerful stimulant of research in universities and elsewhere. It is still a worthy place, but much less a laboratory to be proud of. That, too, is a field ripe for reform.

On the face of things, it would be for the government to put these things to right, just as it has fallen to the government to decide what should next be done about the renegotiation of the British relationship with the European high-energy physics laboratory (CERN) at Geneva. But no doubt from depression, the country's capacity to make decisions seems to have been undermined. The notion that any decision is better than indecision is, of course, dangerous; it provides a licence for foolishness. That is why the scientific community itself should find some way of taking charge of events, such as they are. □

Ballistic-missile defence

US under pressure from Europe to share data

Washington

Now that negotiations on allied participation in the Strategic Defense Initiative (SDI) have begun in earnest, the US government and the Pentagon are coming under strong pressure from the British and West German governments to grant full access to technical know-how gathered in the course of the development as part of the price of overseas participation. But the British government has also raised the possibility that participation might not be worthwhile unless the value of the contracts awarded to British companies and research organizations reaches some predetermined value — \$1,500 million has been mentioned, to the consternation of some administration officials.

The report that the British government is asking for contracts worth at least \$1,500 million appeared last week in the *Baltimore News* newspaper and has been obliquely confirmed. But British officials have said that any figure mentioned at this early stage should be regarded as an "opening bid", rather more an indication of the scale on which the Pentagon should be thinking than as an ultimatum.

Negotiations appear to be at a relatively early stage, with the arrangements for dealing with the security of classified information during the research phase of SDI agreed but not yet formally adopted. One of the points made by British negotiators is that any involvement of British companies as contractors for SDI will involve the British government in supervision and monitoring on a substantial scale, the cost of which would be far from negligible. But the chief concern is that companies or other research entities involved with SDI should not be dealt with as mere research contractors and recompensed on the basis of the time and trouble expended, but that they should be genuine partners in the parts of SDI in which they are engaged.

SDI is likely to centre on fields such as infrared sensors (the basis of boost-phase detection) and signal-processing, in both of which British defence research establishments and associated defence manufacturers have some expertise. One of the British demands is that there should be no restraint and British companies should not be at a disadvantage, compared with US participants, in the later exploitation of developments in which they have been engaged.

A more distant and more difficult issue, raised chiefly by the government of West Germany, is whether governments and national companies participating in SDI should also have access to the later com-

mercial exploitation of technical innovations in which they have not been directly involved.

Even though these questions have not yet been decided, the United States

appears to be in some haste to bring the negotiations to a conclusion. With the sequence of diplomatic meetings planned for next month and thereafter, the bilateral summit in November chief among them, there are obvious benefits in being able to announce allied participation in SDI. No doubt the potential partners have made similar calculations. Just what price will be struck for the political advantage and the technical assistance may be the kind of question the game of poker is designed to answer. □

UK overseas students

Market forces shrink demand

FIVE years after the British government's decision to require overseas students to pay a full "economic" rate for their college or university education, the numbers are still in decline. A report from the Commonwealth Secretariat estimates that the number of overseas students has dropped to some 55,600, a fall of more than 30,000 since the introduction of full-cost fees in 1980, including a fall of 1,000 last year.

Despite a declaration from Commonwealth education ministers meeting in Nicosia (Cyprus) last year that it is important to foster the exchange of students between member nations, the Commonwealth Secretariat notes that exchanges within the Commonwealth are on the decline, but that elsewhere student mobility is increasing. West Germany now accepts 72,000 foreign students and appears to be taking more students from Commonwealth countries no longer able to send as many to the United Kingdom — including Cyprus, Tanzania and Kenya.

The United States remains by far the largest host nation, with some 340,000 foreign students. France has more than twice as many as the United Kingdom (128,000) and the Soviet Union has 82,500. Japan, at present with some 10,000 foreign students, plans to expand this to 100,000 by the end of the century.

The foreign policy implications of providing places for overseas students were recognized by the British government in 1983 when, after considerable pressure from the Commonwealth (most vociferously from Malaysia), the then Foreign Secretary Mr Francis Pym announced a three-year £46 million package to subsidize overseas students. The "Pym package", now entering its last year, singles out certain countries as specially deserving cases—Cyprus, for instance, with a large refugee population, no major university

and strategic importance to Britain — as well as potentially influential individuals from potential trading partners.

The clear determination that the United Kingdom should get "value for money" for overseas students supported financially underlines the economic problem of fostering an exchange between the developed and developing nations. With a brief that the mobility of students between Commonwealth countries is a good thing no matter in which direction the movements take place, the committee responsible for the report suggests that the less developed countries with relatively sophisticated university systems might be encouraged to take increasing numbers of students from countries with few universities.

Not only the supply of university places is affected by economic pressures. Shortage of foreign currency means that the demand is also depressed in countries such as Nigeria and Malaysia, even if the cost of education in host nations is not increasing.

In the immediate future, the current trend in Britain seems likely to continue, as fees are due to increase by a further 5 per cent for the year 1985–86. But for the long term, the committee raises the possibility that student exchanges may not be indispensable for sharing the access to high-level institutions. New information technologies, says the Commonwealth Secretariat, should be considered as an alternative. If the best learning in the world is available at the nearest computer terminal and telephone line, will there be any need for the students to stir from home?

Charles Wenz

"Commonwealth Student Mobility: A Way Forward", is the fourth report of the Commonwealth Standing Committee on Student Mobility (Commonwealth Secretariat, Marlborough House, London SW1 5HX, UK).

Foreign students in higher education in Britain, 1979–80 to 1983–84

	1979–80	1980–81	1981–82	1982–83	1983–84
Commonwealth	43,630	38,718	32,710	28,016	27,370
Non-Commonwealth	44,407	38,912	31,361	28,649	28,238
Total	88,037	77,630	64,071	56,665	55,608
(Commonwealth as % of total)	(49.5%)	(50%)	(51%)	(49%)	(49%)

Source: British Council.

Australian budget

Modest gains for science

Canberra

THE federal government's budget, made public last week, allows modest gains for the government's chief science agencies at a time when the general theme is that of restraint. Indeed, with inflation between 7 and 8 per cent a year, the Hawke government plans to cut its overall deficit to just under \$A5,000 million, some 2 per cent of the estimated Gross Domestic Product for 1986.

According to science minister Mr Barry Jones, the budget for the science portfolio will increase next year to a total of \$537 million, an increase of 6.4 per cent. Within that total, however, there are small but significant shifts of emphasis. The largest single item, the budget of the Commonwealth Scientific and Industrial Research Organization (CSIRO) will be \$A336.7 million which, after allowing for inflation, represents a small cut. No doubt the issue will be reviewed after the appearance of the report on the organization by the Australian Science and Technology Council (ASTEC) expected later in the year.

The chief (if modest) winners from the new budget are the Australian Research Grants Scheme (ARGs), whose budget increases to \$A27.5 million, a rise of 10 per cent. Both the Weather Bureau and the Antarctic Division will have increases of the same size.

Perhaps more significant than these small changes, Mr Paul Keating's budget includes provision for increasing the numbers of students in tertiary education by 20,500. As yet, no decision has been made about the distribution of these places among the three sectors of higher education, but the government announced last week that it had accepted the general arguments of the plan for the reorganization of the Commonwealth Tertiary Education Commission, put forward a few months ago by the commission's chairman, Mr Hugh Hudson. Among other things, Mr Hudson advocated closer integration and liaison among the three sectors.

One unexpected feature of the new budget is that the funds available to the Australian Industrial Development Research Board next year will remain \$A65 million, a reduction of only \$A2 million on this year's budget. It had been expected that these funds would be sharply reduced to offset the plan to introduce, from 1 July next, a 150 per cent tax credit so as to provide manufacturing industry with an incentive to undertake more research and development with their own funds.

For the Australian research community, as for others affected by the budget, the biggest uncertainty for the months ahead is the way in which the economy will respond to the changes that have been made. Mr Paul Keating's plan to engineer

a shift from direct to indirect taxation has now been abandoned. The government was boasting last week of the comparatively rapid economic growth of about 4.5 per cent a year, promising more of the

British research

Bed of nails at last vacated

SIR John Kingman, who has occupied the most conspicuous bed of nails in the British research establishment for the past five years, as chairman of the Science and Engineering Research Council (SERC), leaves this week knowing that his council still has to hammer out a policy for the future. Although impending shortages of



funds sent the council and its staff into an urgent review of priorities last autumn, the resulting "corporate plan" has seemed to the council at its two most recent meetings to be more a list of options than a set of decisions. It will now be for the new chairman, Professor E.J.W. (Bill) Mitchell and the new council (which will have no fewer than eight new members when Mitchell's own place is filled) to decide what lies ahead (see *Nature* 15 August, p.568).

Kingman will become vice-chancellor of the University of Bristol at the beginning of next month. Of the British research enterprise in the past five years, he acknowledges that the British government has kept its promise to keep research spending constant, but that the parallel decline of university budgets had been a serious setback. Now, he thinks, the signs that the rate of emigration of able scientists from the United Kingdom has accelerated again shows that the chickens are roosting uncomfortably.

Politically, there are some hopeful signs. Kingman says, for example, that several Members of Parliament are now more sympathetic than they used to be and conscious that Britain differs from competitors such as the United States and France in attempting to keep science spending constant.

But he also considers that the British

same to come.

But Mr Keating has also been giving solemn warnings that the inflation rate could be sharply increased if wage settlements seek too much compensation for past inflation. Since the budget spending items now announced are fixed in cash terms, their value could be seriously affected by the inflation rate. □

scientific community has been churlish and tactically unwise in not acknowledging more fulsomely that its paymaster, Sir Keith Joseph, Secretary of State for Education and Science, had made a brave political effort to secure extra research money at the expense of student grants at the end of 1984.

Largely for financial reasons, Kingman's spell at SERC has been the most uncomfortable since the reorganization of the 1960s. It has also been a period of indecisiveness. Even major questions such as the council's future commitment to high-energy physics remain undecided and are likely to be out of the council's hands at least until the British government has decided how a renegotiation of the CERN agreement should be opened.

John Maddox

Trees: No news . . .

BRITAIN'S trees are reassuringly healthy, according to a report published by the UK Forestry Commission last week*. In a £40,000 survey during 1984, the commission found that no new phenomena were needed to explain unhealthy trees. Well-known pests, diseases and climatic influences accounted for all such cases. The survey was started because of increasing environmental damage to European forests, particularly in West Germany.

The main aims of the survey were first to assess major tree species to see whether the patterns of decline were similar to those in West Germany and elsewhere in Europe; and second, to relate the symptoms at particular sites to local conditions, especially with respect to atmospheric pollution. The species assessed in the UK survey were Norway spruce, Sitka spruce, and Scots pine.

The survey covered the whole of Britain, and included stands of 35–40-year-old trees likely to remain undisturbed for at least 10 years. Land was divided according to height, amount of rainfall and amount of sulphur deposition. (Sulphur dioxide was the only potential pollutant included in the survey; there is not enough information available on the distribution of rainfall acidity and ozone, for example, to include them in the study.) The Forestry Commission's subcompartment database was used to combine these data with, for example,

Espionage

Soviet scientist accused

A SOVIET scientist, employed at the Arctic and Antarctic Institute of the State Hydrometeorological Commission, made use of research trips abroad to pass on classified information to United States intelligence, according to *Pravda*. Soviet newspapers are usually reticent about reporting criminal or espionage cases, but this particular story was considered worthy of a three-part serial. During the past two years, the Party *agitprop* literature has paid considerable attention to the risks faced by Soviet sailors and others obliged to travel to the capitalist world. The history of the scientist-spy, Yuri V. Pavlov, (code-name Rolf Daniel) was presumably given such extensive coverage to show that even a highly educated Soviet scientist is not immune to temptation.

Pavlov, according to his own statement, quoted in *Pravda*, was the son of a mining engineer and an English teacher. Born in 1935, he studied in Leningrad, specializing in experimental nuclear physics, but abandoned his dissertation work out of boredom. After being involved for some time in a "fantastic project" to create artificial diamonds using nuclear explosions, he drifted out to the Soviet Far East and then, in 1961, took part in an expedi-

tion to Japan, Tahiti, Fiji and Hawaii aboard the doyen of Soviet research vessels, *Vityaz*. Here, *Pravda* implied, Pavlov proved a misfit, being temperamentally unsuited to carry out serious scientific work without "fanfares or laurels".

He returned to Leningrad and for the next seven years he apparently drifted from job to job seeking good pay rather than interesting work.

For a time, he was employed in what was entered in his official work record book as "secret work and documents". In 1969, he went to work for the Marine Register of the USSR and, in his own words, became "one of the central figures in the atomic inspection of the register". Between 1977 and 1980 he travelled abroad to meetings of an international working party on safety regulations for nuclear ships, visiting London (four times), Genoa, Ottawa and Hamburg. Finally he left this job however, feeling that it was "too narrow" for him and in the autumn of 1981 began working at the Arctic and Antarctic Institute, in order that he might avail himself of the opportunity of foreign travel aboard the research ship *Professor Vize*.

During his travels, Pavlov stated, he was recruited by West German intelligence and the Central Intelligence Agency. The *Pravda* narrative traces out the course of his contacts in foreign ports, the "spy kit" issued to him, the efforts of Soviet counter-intelligence to track him down and his final confession and repentance and the imposition of an (unspecified) appropriately severe sentence" (presumably the death penalty) by a military court.

The exact data which Pavlov allegedly handed over to the West are left vague, but a tailpiece entitled "instead of an epilogue" puts the story firmly in the context of US/NATO "aggression" as revealed by "star wars", psychological warfare and disinformation.

Ceaseless vigilance, says *Pravda*, is the "principal weapon" in this ideological war. Pavlov, on the contrary, exhibited the "lightmindedness" of "ideologically unstable people", who "live in a vacuum" and who must be excised from Soviet society as a "foreign body".

Perhaps the most telling summing-up of Pavlov's character faults, however, comes not in this tailpiece but in the body of the narration, where he is accused of "Manilovism". This word, drawn from the name of a character in Gogol's *Dead Souls*, does, indeed, typify the shiftlessness and lack of stability which, presumably, Pavlov exhibited, but it is a word that has not recently been much in vogue in Soviet character-analysis, having been one of Stalin's favourite epithets of condemnation.

Vera Rich

Nuclear waste

EPA regulates at last

Washington

THE US Environmental Protection Agency (EPA) has at last issued regulations that it wants to see enforced at high-level radioactive waste disposal sites. But the "final" standards are unlikely to be the end of a long inter-agency dispute on how the regulations will be enforced, and there seem likely to be many opportunities yet for opponents of geological disposal to mount legal challenges.

EPA has come out on the wrong side of an argument with the Nuclear Regulatory Commission (NRC) over regulation of disposal sites, the upshot of which is that EPA can only implore NRC to comply with a list of assurance requirements designed to ensure that there will be no significant leakage of radioactivity for 10,000 years after disposal operations are completed. The practices at the disposal sites while they are still in operation will be regulated by NRC, whose standards are different from those promulgated by EPA.

The need for a permanent resting place for US nuclear waste has become urgent in recent years. In 1982, a report from the Congress's Office of Technology Assessment warned that some power stations might have to close down unless provision was made for disposing of high-level waste, mostly used fuel rods, now accumulating in ponds at power station sites. Given the tarnished public image that nuclear power already suffers in the United States, that was an option to be avoided at all costs.

Responsibility for selecting and operating a disposal site rests with the Department of Energy, which is now engaged in processing some 20,000 comments on its proposal to carry out on-site investigations at three possible sites for a high-level waste repository. By the end of the year, the department hopes to have confirmed its previous choices and to have issued final environmental assessments. A final decision will then be made by the President and Congress.

The standards that EPA has now issued limit exposures to members of the public to 25 millirems to the whole body, 75 millirems to the thyroid and 25 millirems to any other organ. There is also a waiver whereby the EPA administrator can raise the 25 millirems limit to 100 millirems if he sees fit or 500 millirems for a one-off dose. And the standards limit projected releases of radioactivity to the environment for 10,000 years; EPA estimates that compliance with the containment requirements means that there will be no more than 1,000 cancer deaths over the entire 10,000 years considered. Tim Beardsley

... is good news

tree species and age, to provide the basis of stratified sampling schemes.

No needle yellowing, such as that found in Norway spruce in West Germany, occurred in Britain. A strong regional effect for crown density and needle age could have been caused by local environmental factors or by bias among surveying teams. Scots pine and Norway spruce hold their needles for longer at higher altitudes but had less dense crowns where rainfall is higher. Scots pine held its needles for longer, but Sitka spruce had less dense crowns, in high sulphur deposition zones. None of these signs of sickness was caused by previously unknown phenomena.

Although the news is good for Britain's trees, optimism should only be cautious. A single survey has limited use in detecting changes in forest health, but patterns should emerge with subsequent re-surveys. West German surveys cannot easily be compared with the UK data because of differences in management, altitude, climate and, particularly, age of trees (old trees are more susceptible to pollution damage). As the trees in the UK survey grow older and the local levels of pollution vary with time, patterns of damage, if they exist, will emerge.

Maxine Clarke

*Forestry Commission Research and Development Paper 142 Forest Health and Air Pollution: 1984 Survey £1.40.

Nuclear India

New reactor "not for bombs"

New Delhi

INDIA has denied reports that the country's largest research reactor, commissioned on 8 August, is intended to breed plutonium for bombs to counter Pakistan's nuclear threat. A spokesman for the Department of Atomic Energy (DAE) says that the 100-MW DHRUVA reactor at Bombay is capable of producing annually 30 kg of plutonium which will be outside international safeguards, "but it is not a dedicated facility". He says that like all uranium-fuelled reactors in the world, DHRUVA will breed plutonium, but is not linked with any weapons programme.

India is not in fact dependent solely on DHRUVA for plutonium if it did decide to go nuclear. It has been reprocessing spent fuel from CIRUS, an ageing Canadian-built 40-MW reactor operating in Bombay, since 1963. CIRUS was the source of plutonium for the nuclear test in Pokran in 1974. Another 100 kg of plutonium, also from CIRUS, was used to make the Pu-U carbide fuel for the Fast Breeder Test Reactor (FBTR) which is nearing completion at Kalpakkam near Madras.

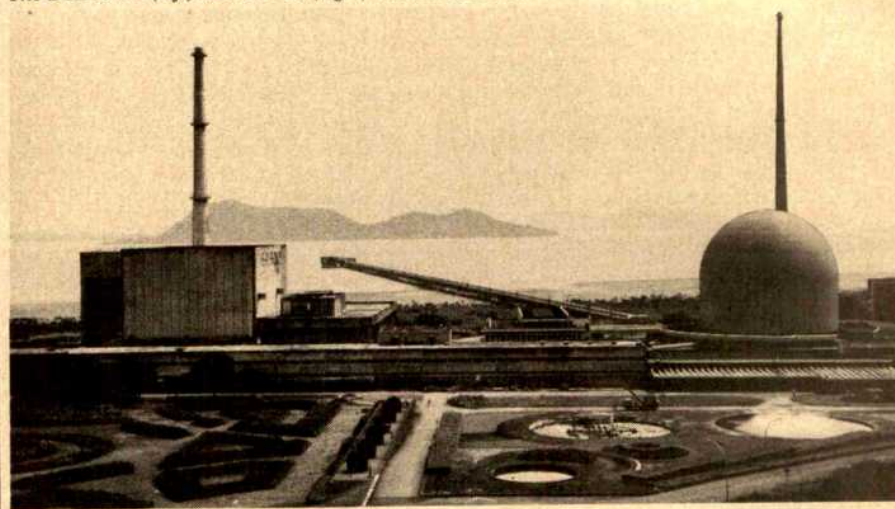
Neither CIRUS nor the first 230-MW unit of the Madras Atomic Power Plant (MAPP-I) which has been operating since 1983 is under safeguards; these apply to the US-built power plant in Tarapur, and the two atomic power reactors in Kota, Rajasthan. While Tarapur fuel has not yet been reprocessed, every gram of plutonium produced at Kota has been accounted for. India's current stock of plutonium is estimated to be at least 200 kg. DAE officials point out that it is not from lack of plutonium but by a political decision that India has not been making bombs.

Four days after DHRUVA became critical, DAE commissioned MAPP-II, the second power plant in Madras. This "90 per cent indigenous" power plant, together with DHRUVA, which will replace CIRUS in five years, will no doubt add to the stockpile of unsafeguarded plutonium which DAE says is required to fuel the fast breeder reactors India plans to build in the 1990s. The FBTR, similar to France's RHAPSODIE, is expected to become critical in September and India is well advanced in the design of a 500-MW prototype commercial breeder.

According to DAE, the DHRUVA reactor will be used to produce isotopes for agriculture and medicine, besides research on solid state which requires a higher flux of neutrons than is available at CIRUS or APSARA—a one MW swimming pool reactor built in 1956 using British supplied enriched uranium. DHRUVA, which uses natural uranium fuel and a heavy water moderator, is designed for a flux of 10^{14} neutrons at the centre of the

core, and is thus comparable to the NRX reactor of Canada. DHRUVA will, however, operate at full power only after DAE has solved the serious problem of vibration of fuel rods and ensured complete safety of the interlock systems. When one of the interlocks failed two days before the reactor went critical, four ton-

The DHRUVA (left) and CIRUS (right) nuclear reactors.



nes of heavy water worth £0.75 million were spilt. MAPP-II, which went critical without a hitch, is expected to be synchronized with the power grid in November. With the commissioning of the country's sixth nuclear power reactor, the nuclear contribution to India's total power capacity will reach 1,320 MW, or four per cent. The target, according to Dr Raja Ramanna, chairman of the Atomic Energy Commission, is 10,000 MW by the year 2000.

K.S. Jayaraman

Soviet alcoholism

Drunk driver stripped of degree

THE Soviet Union's new campaign against alcohol abuse has cost at least one scientist his degree, and the lifelong financial benefits that go with it. The legal provisions, introduced in 1976, allowing those found guilty of conduct unbecoming to a Soviet citizen to be deprived of their degrees, has so far been used only in a handful of cases involving Jewish refusniks.

The penalty has now been imposed in the bizarre case of Boris Samarin, a former employee of Leningrad University, who is just completing a four-year sentence for causing the deaths of six companions while driving under the influence of drink.

The accident, which took place in the small Karelian village of Suoyarvi, involved members of the Leningrad University Karelian Geophysical Expedition, headed by Samarin. On the way back from the expedition, Samarin was travelling in the cabin of the expedition truck, and both he and the driver, Viktor Kolesnik, were drinking heavily. When the truck ran off the road into a swamp, all six people in the body of the truck, three girl students, two women scientists and the small son of one of the latter, were drowned, Samarin and Kolesnik escaped to face the legal consequences.

Court cases are normally reported in the Soviet press only to illustrate some facet of current policy, and it was only last August, more than three years after the

crash, that the Moscow *Literaturnaya Gazeta* carried an extensive article on the incident, pointing out that Samarin was well known to be a heavy drinker, that members of the hydrogeology department were reluctant to go on expeditions with him (indeed, his expeditions regularly included a number of "dead souls", who figured in the official report but who had never actually left Leningrad), and that, nevertheless, no apparent efforts were made to curb his drunkenness nor to take the organization and running of expeditions out of his hands. How, asked the *Literaturnaya Gazeta*, could such a situation arise?

A few weeks later, the newspaper printed a reply from the rector and the secretary of the Party Committee of Leningrad University. This, in effect, pointed out that the unfortunate incident had taken place three years previously, and that as a result of the tragedy, procedures relating to university personnel and to the organization of expeditions had been considerably revised.

Last month, as a postscript to the whole affair, the paper noted that it had received a letter from Dr G.M. Nesmeyanov, the head of the Higher Attestation Committee, VAK, the body responsible for higher degrees, saying that Samarin had now been deprived of the degree of Candidate of Geological and Mineralogical Sciences.

Vera Rich

Environmental organizations

Workings of Greenpeace

THE boats of Greenpeace both symbolize and implement the direct action campaigns by which that group enters some of the world's most highly publicized environmental controversies. Greenpeace's willingness to establish a physical if non-violent presence gives it an "extreme but not extremist" character. "People want to see effective environmental campaigning for their money", says one spokesperson, pointing to press coverage of the group's activities as one measure of fulfilment of that promise.



Although environmental groups sometimes formally pool their resources, such as in the consortium fighting the US sanctioning policy on Japanese whaling, the strength of the environmental movement, according to Peter Wilkinson, member of Greenpeace's international board, lies in its diversity. Greenpeace takes a radical stance amid this diversity, though not as radical as that of groups espousing violence. Alan Pickover, Greenpeace international coordinator for marine animal campaigns, says that the group's position on the outer fringes often serves to push governmental compromises towards the middle ground of environmental opinion, which would not happen if conservative groups such as the Royal Society for the Protection of Birds were the primary environmental voice.

The sinking of *Rainbow Warrior* has not changed Greenpeace campaign priorities, although the loss of the £250,000 investment has resulted in a scramble for campaign funds and rearranged schedules for other ships. Greenpeace offices worldwide, says Mr Wilkinson, have been inundated with messages of support, although the level of contributions has not yet been assessed.

With the loss of *Rainbow Warrior*, which was based in the United States, Greenpeace is left with only two large ships. The older of the two, *Serius*, has just left an aborted attempt in Iceland to stop that country from killing 200 whales next year for "scientific research". Only the first instalment has been paid on the ocean-going tug *Greenpeace*, now on its way to Mururoa and Antarctica. Green-

peace had bought this vessel before *Rainbow Warrior* was sunk and will have to pay the remaining \$360,000 over the next three years. The organization also owns a river-going vessel bought with publicly raised funds and equipped to detect pollution as well as a hot air balloon held by the East Germans ever since it inadvertently sailed over the Berlin Wall.

Greenpeace has offices in 15 countries. For an office to have a vote on Greenpeace policy, it must not only be self-sufficient but also contribute funds to international campaigns. Some of the six fledgling offices have simply not had the time to mature; others, such as the French office, are important for political reasons even if they do not make much money. Every fall, representatives from these 15 countries meet to discuss policy and international campaign budget issues. Their decisions determine the organization's priorities for the coming year as well as setting the official Greenpeace viewpoint on any given issue. An elected international board of five is responsible for monitoring as well as for emergency decisions in the course of the year.

Greenpeace offices worldwide are organized in different ways depending on individual countries' needs and laws. Only in the United States does Greenpeace maintain full-time government lobbyists because policy-making there is amenable to such pressure tactics. In Europe, on the other hand, lobbyists concentrate on international conferences. Each office supports at least one expert in each of the primary areas of Greenpeace activity, but campaign strategy in any area is settled at the international rather than the national level.

International campaign proposals fall into four categories: nuclear; marine animals, which includes fisheries; chemicals; and terrestrial animals. Before the annual meeting, the campaign coordinators responsible for these areas, armed with figures of the available funds, work out proposed budgets with the campaign directors in each area. Greenpeace commissions technical reports and also uses experts on each animal or topic at issue to determine where the greatest needs lie. The compromise decisions that are made at the annual meeting tailor the proposals to stay within the expected income for the coming year. In 1985, 29.1 per cent of direct action money was spent on marine animals, 22 per cent on nuclear, 12.4 per cent on chemicals and 1.1 per cent on terrestrial animals (mostly kangaroo pelts and meat). The high cost of the boats eats up most of the remaining 35.4 per cent; a three to four-day action in the North Sea, for example, costs roughly \$20,000.

Trouble can arise because of unexpected losses such as *Rainbow Warrior* or if offices do not raise the "notional money" they had pledged and which was divided up in the annual meeting setting out goals for that year. After the budgets for running national offices are deducted, the remaining funds from all the offices are pooled to cover Greenpeace's international campaigns. The estimated income for 1986 is \$10 million, with 80 per cent of that available for campaigns. According to one spokesperson, Greenpeace income has been increasing at roughly 10 per cent a year, with the average donation \$10-100 and virtually no donation over \$1,000. There is no extra room in the budget. According to Mr Wilkinson, Greenpeace's bank balance after campaigns is only \$300,000, which represents a dangerously small margin of leeway.

Elizabeth Collins

AIDS

US company rejects UK decision

THE UK Public Health Service (PHS) evaluation of test kits that detect the presence of antibodies to the AIDS (acquired immune deficiency syndrome) virus in the blood has been criticized by one of the companies that did not come out well from the study (see *Nature* 8 August, p.474). Of the five companies whose kits were evaluated, Wellcome Diagnostics (UK) and Organon Teknika Ltd (The Netherlands) were selected for the second stage of the evaluation, testing by the blood transfusion service. By October, all blood donated in the United Kingdom will be screened for the presence of the AIDS virus, probably using one of these kits.

But Abbott Laboratories Ltd, US manufacturers of one of the unsuccessful kits, is not satisfied with the results of the PHS evaluation. The company is lobbying government officials and physicians in the

United Kingdom to persuade them that the results of the survey are "preliminary and misleading". Kits currently available on the US market, which include Abbott's, are almost completely efficient at detecting AIDS virus antibodies.

Whether Abbott's complaint is justified will not be known until PHS publishes the details of its evaluation early next month. If Abbott's test is not one of those recommended by the health department to the health authorities for use in the transfusion service, the company will lose out in a business that is worth £5-10 million a year. Abbott claim to have a prototype kit that detects viral antigen, a test which would enable much earlier identification of people at risk to or carrying the AIDS virus and which is recognized to be the next step in the control of the disease.

Maxine Clarke

Animal intelligence

SIR—According to Geoffrey Hall's review of *Animal Intelligence* (*Nature* 25 July, p.306) "... modern experimental studies of animal learning... have found precious few differences between species that can be unambiguously interpreted as being the result of differences in intelligence. Macphail concludes that we might as well accept ... that extant non-human vertebrates do not in fact differ in intelligence". I would like to support the reviewer's comment that this conclusion is a condemnation of the skills of modern experimenters rather than an accurate judgement of the intelligence of animals. There is surely something very wrong with studies that show no unambiguous differences in intelligence between a frog or a chameleon — or even a sheep — and a sheepdog or a chimpanzee.

Very complex behaviour patterns can be built into the simple nervous system of an insect, a honey-bee can quickly learn to find its nest by what must be a quite comprehensive imprinting essential to the success of its way of life. The ability of a rat to learn its way through a maze may not have much relevance to its intelligence. Young rats may be programmed to learn complex and growing mazes of tunnels.

The primary function of intelligence must have been to improve the chance of survival or it would never have evolved. For a specific way of life in an unchanging environment, a built-in behaviour pattern that has been optimized by natural selection over 5 million years — a typical species lifetime — is not easily improved by an individual creature. Improvement must require occasional replacement of part of the built-in behaviour pattern by an anomalous behaviour locally more successful than the five-million-year optimum. This in turn requires three things. The first is a good memory of events in the individual's life, such as stumbling on a novel source of food in an accidental excursion into a normally avoided environment, or of a narrow escape by novel means from a novel hazard. The second is the ability to recognize the value of repeating the experience. The third is the ability to override the built-in behaviour pattern in order to do so.

The enormous gap between human and even the greatest animal intelligence could mean that we ought to use different words for them, but I believe that the difference is quantitative rather than qualitative. We passed a vital threshold when we became bright enough to carry throwable chipped stone weapons. A group so armed would have become very unattractive to the big predators, and survival from then on must have depended on intra-specific competition for food-bearing territory between groups. This did nothing to make us tolerant of groups of strangers but a lot to increase our intelligence. A second

threshold was passed when we developed language. The value of this in original practical thinking is often exaggerated, but its value in transmission of thought and knowledge and hence in inter-group fighting was overwhelming.

The value of intelligence will be much increased if the environment undergoes a large and permanent change. At the end of the Cretaceous period, the Earth seems to have been struck by a large meteorite and the resulting dust clouds may have persisted long enough to eliminate many species of plants and animals over large areas. The survivors will then have been thrown suddenly into a changed biological environment in which many of the less adaptable species may later have died out. Then those species, for example the early mammals, that could diverge from their built-in behaviour, and learn from their own experience and from novel behaviour of parents or other conspecifics, would have had a great and sometimes overwhelming advantage over those with rigid behaviour patterns.

To measure intelligence we must do

more than measure memory. The ability of a rat to learn a maze is a good test of memory, but could be using the same kind of built-in learning pattern as that of the honey-bee. It would be better to give the rat a chance of some lateral thinking by, for example, painting a particular scent on the wall in the middle of each straight section on the side to which the rat should turn at the next fork. Increased speed of learning another maze with the same cues could hardly result from any built-in pattern. Similar problems could presumably be arranged for a goldfish, a tortoise, or more expensively a chimpanzee.

To summarize, I believe that there are differences in intelligence, which must in principle be observable, between different vertebrates, and that there are understandable reasons why these should have arisen. Furthermore, in testing for intelligence, I believe that one should try to distinguish between memory and the ability to notice correlations between experiences, and should look for ability to override normal or usual types of behaviour.

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PhD theses

SIR—A PhD is supposed to be a training in research. Normally this process is conducted over a period of three years, financed by either government or industry, and involves three years' supervision by a university teacher, to which must be added the cost of equipment both capital and consumable, and technical assistance over the requisite period. The return for the many thousands of pounds of outlay per candidate is a thesis on a university library shelf and a piece of paper for the successful candidate which hopefully will enable him or her to command a higher salary than he or she could otherwise have expected.

While it is true that theoretically theses can be consulted in university libraries, in practice this is rarely done. References to PhD theses occasionally appear in the scientific literature, but usually only when an author refers to his own unpublished thesis.

Research is not recognized as having been brought to a conclusion until it has appeared in print in a recognized scientific journal. An essential part of training in research should be the preparation of the work for publication as a scientific paper. A PhD should not be awarded until there is evidence that the major new contributions to science have been published or at least accepted for publication in an appropriate reputable journal. If the material is not suitable for publication then it was not necessary for it to be done in the first place. If there are a large amount of basic data for which there should be ready access, then this could be

deposited in suitable archives as is already the practice with learned societies.

It must surely be time for the antiquated PhD thesis system to be thoroughly overhauled. The stupendous waste of resources cannot be justified at this time. It cannot be beyond the wit of man to devise a system more in keeping with present needs.

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Modest disclaimer

SIR—John Maddox's readable account (*Nature* 11 July, p.101) of the experiment that T. Corle and I made to measure Maxwell's displacement current inside a capacitor as it is being charged does us too much credit. Although we may have been the first to measure the profile of the magnetic field between the capacitor plates, we were not the first to measure the total magnetic flux.

That experiment was done almost 60 years ago by M. R. van Cauwenberghe (*J. Phys. Radium* 10, 303; 1929). The title of that work, "Vérification Expérimentale de L'Équivalence Électromagnétique entre les Courants de Déplacement de Maxwell et les Courants de Conduction", aptly expresses the results of a very pretty measurement which used an iron-core, toroidally-wound solenoid to measure the magnetic flux through the central portion of a capacitor.

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Rates of galactic star formation

A remarkably simple argument successfully accounts for the rate of star formation in different galaxies. The snag is that the timescale is uncomfortably short.

How could star formation be infectious, or autocatalytic, "catching" in the vernacular? Simple prejudice says that such a mechanism is too anthropomorphic to be real. But the simple sight of a spiral galaxy such as that of the Milky Way will show that the pattern of star images, but especially that of new stars, has a clumsiness that suggests their congregation. The mass of stars in the Orion nebula argues the same case. The objective way out of this dilemma is to follow Schmidt (originator of the fast sky-camera) in supposing that the rate of star formation is somehow a function of the density of the raw material, interstellar gas. Obviously the functional relationship cannot be linear, for then the ratio of stellar mass to that of the immediately surrounding gas would be uniform in space. Schmidt (*Ap. J.* 129, 243; 1949) suggested that the rate of star formation might be proportional to the square of the density of interstellar gas, or something like that.

Michael A. Dopita, from the Mount Stromlo and Siding Spring Observatories, who has a better autocatalytic idea, points out (*Ap. J.* 295L, 5; 1985) that no relationship of the type proposed by Schmidt can explain why the Smaller Magellanic Cloud has only one fifth as much mass locked up in stars, per unit mass of gas, as has the Larger Magellanic Cloud. Infection, autocatalysis or positive feedback in star formation is his solution.

Suggestions that star formation may stimulate further star formation are far from novel. The mechanics of such a process are easily described. Newly formed stars will heat and even ionize the immediately surrounding interstellar medium, while those massive enough to run through their evolution quickly to form supernovae will add hot material to their environment. The result is that the interstellar medium is filled with hot gas in a set of circumstances, far from equilibrium, in which the colder material in the environment is compressed into clumps that may be massive enough to collapse under their own weight into proto-stars and eventually stars. Dopita refers to several explicit accounts, going back to the late 1970s, of how such a mechanism may account for observed properties of galaxies.

His own contribution is what he calls a "law of star formation" to replace that originally suggested by Schmidt. Time will tell whether it provides a sufficient ex-

planation of the variation of the rate of star formation from one galaxy to another as well as from place to place within a single disk-shaped galaxy. But its immediate virtue is the simplicity of its argument.

To make sense, a law of star formation must link the observed rate of star formation with something else that can be observed. Dopita settles for the surface brightness of neutral hydrogen of a galaxy, or of a part of it, which the radioastronomers are able to measure directly. The essential link between these quantities is simply that of kinetic theory, that the mean squared velocity of the gas is proportional to the pressure.

Dopita's argument then starts from the assumption that the interstellar gas pressure is a linear function of the two quantities representing the mass density of stars and the rate at which that variable changes with time as a consequence of star formation. In gas-rich galaxies (such as both the Magellanic clouds) but also in other circumstances, the rate of star formation will be the dominant determinant of interstellar gas pressure. In those circumstances, Dopita concludes, the total rate of star formation in a galaxy should be proportional to the product of its total mass and the surface gas density (in grams per cm^2 , for example).

The prescription seems to work remarkably well. At least on a log-log plot (of the rate of star formation and the product of total mass and surface gas density), both the Magellanic clouds, our own galaxy and a score of other gas-rich galaxies lie within a factor of less than 10 of a straight line, which is probably as much as can be expected for quantities such as the rate of star formation whose measurement is more a matter of inference than of observation. Certainly the result will be taken as an explanation of why the Smaller Magellanic Cloud has many fewer stars than its companion satellite galaxy — its mass is smaller.

The more typical case in which the interstellar gas pressure is a function both of the rate of star formation and of the density of pre-existing stars leads to a more complicated but still simple relationship. One necessary step in the argument is to estimate the fraction of the material incorporated into new stars which remains locked up in them. They must be objects not so massive that they form supernova explosions. The result, however, is a first-order linear differential equation for the

surface density of gas as a function of time. Integration leads directly to an expression for the surface gas density as an exponentially decreasing function of time which, significantly, does not decrease to zero but, rather, to a non-zero value. In other words, even in the galaxies most productive of new stars, there will always remain a certain amount of gas that is not incorporated into stars.

Again, the agreement with observation is good. The surface density of gas as a fraction of the total surface density of a galaxy should be a straight-line linear function of the ratio of the star-formation rate to the total mass of stars, either for an entire galaxy or for part of it. In the sample of galaxies Dopita uses for the comparison, the only two galaxies that do not lie near the curve are, in a sense, exceptions that "prove the rule" — other evidence suggests that they have recently accreted gas from some extragalactic source, and are thus artificially young.

Dopita himself says it is remarkable that "such a simplistic" model should give such a good fit. He also points to what may be the most uncomfortable consequence of the argument, the short timescale for galactic evolution implied by the constants in the model, determined by the best fit of the observations with the predictions. Most galaxies, on this basis, are using up their gas content on a time scale which is short, on the order of 1,000 million years, compared with the time scale of the Universe. Dopita is again quick to point out that this difficulty has arisen previously, most notably in the work of the late B.M. Tinsley and her colleagues but specifically in work such as that of R.C. Kennicutt (see *Ap. J.* 272, 54, 1983), in which ultraviolet emission from hydrogen is used as a direct index of star formation.

On the face of things, this implies that the history of the present era of star formation in isolated disk galaxies is shorter than has previously been assumed and that, for some galaxies, the renewal of the stellar population may have only a short way to go. The puzzle is an important conundrum whose resolution is nevertheless not entirely beyond the bounds of possibility. Plainly the Dopita law does not take account of all the possibilities. But any explanation can only further complicate the difficulty of learning enough about the evolution of galaxies to rack the question of the evolution of the Universe as a whole.

John Maddox

Palaeoanthropology

Palaeobiology and age of African *Homo erectus*

from Eric Delson

FINDS of ever-older human relatives and potential ancestors have caught the public's imagination in recent years. Kenyan fossils newly described on page 788 of this issue by Brown *et al.*¹ could help to swing the pendulum of public interest back toward younger fossils belonging to species of our own genus, *Homo*. Since 1969, Richard Leakey and his colleagues have recovered human and other fossils from deposits along the eastern shore of Lake Turkana in northern Kenya. Now, a group has crossed to the west side of the lake; early work has yielded a fossil already touted in the press as a "strapping youth" whose bones will shed yet more light on human ancestry².

The new fossil has been identified as a representative of *Homo erectus*, the extinct species first named *Pithecanthropus erectus* or "Java Man" by Dubois in 1894. Skulls of many individuals of this form are now known from Java and China, mainly estimated to date between 1.2 and 0.25 Myr old^{3,4}. It is widely accepted that populations similar to *H. erectus* were directly ancestral to the earliest members of the living species *H. sapiens*, although the exact timing, geography and mode of transformation are still controversial. Unfortunately, limb bones are scarce in the Asian sites, and some of the most complete have been questioned as to their specific identification⁵.

That is the main value of the new Kenya fossil — it preserves almost all of the skeleton of a 1.6-Myr-old individual who can be sexed (as male, by pelvis shape) and aged (12 years, from tooth eruption timing). Fossils identified as *H. erectus* have been known in eastern Africa since 1960, when Louis Leakey reported a skull cap from Olduvai which matched those from Asia. Moreover, Olduvai and other sites have yielded partial limbs and pelvises attributed to *H. erectus*, but none has been associated with a well-preserved cranium, always rendering precise taxonomic identification doubtful. The West Turkana fossil (catalogued as KNM-WT 15000) preserves most of the skull and lacks only the extremities and forelimbs (the right humerus remains). The team expects to find additional portions of the skeleton with further excavation, but this degree of preservation is otherwise unknown before the Neanderthals of 40,000–100,000 years ago. By comparison, 'Lucy', the 3-Myr-old partial adult female skeleton of *Australopithecus afarensis* recovered by Johanson⁶, has a lower jaw but only bits of the skull; many fewer ribs and vertebrae; the upper and lower leg bones of only one

side of the body; but does preserve most of the two forearms and left humerus missing in WT 15000.

What will this new information tell us about how *H. erectus* lived? That is still hard to say, but one point much emphasized is the apparently great height of the individual: 1.6 m (5 ft 5 in) at death, perhaps 1.8 m (6 ft) if adult¹. Brown *et al.*¹ have moderated this claim, especially because the skull of *H. erectus* was lower than that of the modern *H. sapiens* whose bones were used to develop the height regressions; the regression estimates should consequently be reduced.

Previous estimates for the height of *H. erectus* ranged between 1.55 and nearly 1.8 m^{7,8}. They were usually based only on the length of the femur (thigh bone) and



Locations of major *Homo erectus* sites. There is controversy over whether the European and Baringo fossils should be allocated to *H. erectus* or *H. sapiens*¹².

did not consider skull height differences. Thus, the WT fossil might not have been especially large for this species to begin with. Moreover, additional cavils need to be evaluated. Modern humans show an adolescent growth spurt, especially in males. If such a spurt existed in *H. erectus*, it might have led to a height greater than the estimated 1.6 m, had the specimen lived longer.

Similarly, it is unclear that dental eruption timing then was the same as it is today; T. Bromage and C. Dean have questioned the equivalence of such timing in *Homo* and *Australopithecus* species based on counts of perikymata (developmental lines in tooth enamel)⁹. On the other hand, if most *H. erectus* individuals were rather shorter than WT 15000 (which is less likely), it is possible that this individual was atypically tall or died young because selection acted against his continued growth.

Palaeoanthropologists will probably be more interested, however, in the insights that this fossil will provide into the

patterns of growth and inter-individual differences among earlier humans. As evidenced by both dental eruption and epiphyseal fusion stages, WT 15000 was clearly juvenile. Yet Brown *et al.*¹ note that the face is larger and more massive than that of an adult *H. erectus* skull from the east side of Lake Turkana they have just described¹⁰. That skull, ER 3733, is widely accepted as female, so that its relative gracility compared with WT 15000 is not surprising (except for the age of the latter). It would be interesting to compare WT 15000 with ER 3883 (ref. 10). Some authors think the latter was a male (ref. 8) but I consider it another female and the large but stratigraphically younger Olduvai OH9 to be the male in this sexually dimorphic species.

This question leads inexorably to the two main recent foci of attention on *H. erectus*: does the species illustrate stasis or phyletic change through its 1.5 Myr span; and are the African forms really the same species as the Asian? One of the most important types of information that bear on both these questions is accurate ages for the fossils. Although Pope³ and other workers have recently argued that the oldest Asian *H. erectus* are often significantly younger than previously thought (1–1.2 Myr at most), careful re-analysis of several East African geological sequences has clarified the age of the earliest supposed members of the taxon there. Also in this issue, McDougall *et al.*¹¹ on page 792 present new data on the age of one major Turkana Basin marker horizon, the Okote Tuff complex. They report that the basal levels of this 10–15-m-thick unit date to no more than 1.64 Myr. The pumice fragments dated have a composition different from that of the enclosing tuff, suggesting reworking upwards of older pumice into the slightly younger tuff. Brown and Feibel¹² on page 794 offer further local correlations and review other dates which indicate that it is not much younger (>1.52, probably >1.6 Myr). In addition, they determined that a tuff slightly below the horizon of WT 15000 is equivalent to one of two newly defined tuffs on either side of the Lower Okote, yielding the age of 1.55–1.65 Myr for the skeleton.

These dates and others in the Early Pleistocene (through to perhaps 1.0 Myr) make it simple to begin the study of temporal variation in *H. erectus*. But as noted by Pope³, dates in Asia are still highly uncertain and younger African fossils (especially in the Maghreb) are also of questionable age. Thus, although studies such as those of Howells¹³ and Rightmire¹⁴ have suggested that variation through time is not continuous or even directional in *H. erectus*, Wolpoff¹⁵ has recently argued strongly for a gradualistic view of temporal variation and change in this species. His comparisons of 13 cranial characters across specimens grouped into three time ranges are superficially persuasive, but there are too many potential problems

for his analysis to be convincing. Several individual fossils are of questionable identification, sexual variation is not considered and the raw data are not available to see the precise effects of these factors. Equivocal age determinations and the use of time bands rather than age estimates that would permit regression analysis also disturb me. As Wolpoff notes, it has been "excruciatingly difficult to test the punctuated equilibrium model in the fossil record", not least because of methodological disagreements among researchers.

An examination of Wolpoff's list of specimens reveals that whereas East African and Indonesian fossils dominated the 'early' and 'middle' *H. erectus* time groups, the sample from Zhoukoudian, China dominates the 'late'. Some authors have recently questioned whether such comparisons are as meaningful as we might like—are the African fossils indeed members of a palaeobiological species *H. erectus* as defined from the unique characteristics of the Asian specimens defining this taxon? Howell¹⁶ reviewed the consensus position of geographical identity some years ago, providing a detailed descriptive definition without specific regard for the derived versus the conservative nature of the characters discussed. Rightmire^{17,18} has been the most vocal recent proponent of this view, arguing that several uniquely derived characters define *H. erectus* in Asia and Africa without recourse to the arbitrary use of temporal gaps or rubicons.

On the other hand, Wood¹⁹ and Andrews²⁰ argue strongly that African fossils assigned to *H. erectus* share only conservatively retained features with Asian members of that species, not any of their derived characters. Thus, they imply the need for recognition of a new species to receive African fossils possibly intermediate between *H. habilis* and *H. sapiens*. Stringer²¹ is more cautious, but agrees with

the lack of shared derived features in specimens from the two regions; he notes further that if *H. erectus* were to be defined broadly enough to include all of these fossils, most derived conditions seen in the younger Asian specimens (especially those found in China) would reject the concept of stasis in favour of definite change over time.

Palaeoanthropologists continually make the claim that more fossils will help answer pressing questions, and field workers who find them reap well-deserved rewards. If WT 15000 is any example of the quality of

material to be expected in future years from the West Turkana sequence, there will certainly be much grist for our mills. But the milling process must include careful and probing analyses of all the available fossils, matched with a willingness to test both revisionist and entrenched hypotheses and to embrace new interpretations, if we are to take full advantage of the hard-won fossils. □

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Genetic diseases

Probe for muscular dystrophy

from P. Michael Conneally

DUCHENNE muscular dystrophy (DMD) is probably the most common X-linked disorder in man, affecting one in 4,000 males, the disease being transmitted by carrier females. Sisters of affected boys have a 50 per cent risk of being carriers, but there has been no reliable test for prenatal diagnosis or for carrier females, although serum creatine kinase levels are elevated in approximately 70 per cent of carriers¹. The locus for DMD is in the middle of the short arm of the X chromosome (Xp21) and two DNA probes that flank the locus have been reported within the last two years², but unfortunately they are quite far away. Elsewhere in this issue Monaco *et al.*³ describe the successful cloning of a DNA fragment that detects a deletion which is in or very near the DMD gene. This is a major breakthrough that forms the basis for much more accurate detection of females carrying the gene and for prenatal diagnosis, and that will also help us to an understanding of the aetiology of this lethal disease.

About 600 males affected by DMD are born annually in the United States alone. It is a progressive muscle-wasting disease with onset before the fifth birthday; victims are confined to wheelchairs by their early teens and usually die before they reach their early twenties. In families with one affected boy, 50 per cent of his sisters are likely to carry the gene, so about the time the affected boy dies, his sister may be giving birth to another affected boy. The new probes will mean that accurate prenatal diagnosis and carrier detection will now be available for families with affected children, and so should lead to a substantial decrease in the burden the disease places both on families and health services.

The method that Monaco *et al.* have used, which is reported in detail in a separate publication⁴, involves a very clever technique that can also be used for a number of other genetic disorders. The method allows the cloning of DNA missing from patients homozygous (or

hemizygous in the case of X-linked traits) for a chromosomal deletion. The principal of the technique is to hybridize excess DNA from the patient with the deletion to DNA from normal humans: the DNA that fails to hybridize is consequently enriched with sequences from the region of the deletion. As an extra precaution, in the case reported here, the DNA from a boy with DMD (and other problems, like retinitis pigmentosa) and a small deletion in the short arm of his X chromosome was hybridized to DNA from cells with an excess (five) of normal X chromosomes. This method is likely to be generally useful in cloning DNA containing the affected gene in specific genetic disorders involving chromosome deletions or translocations. In the past few years, it has been possible to go from genomic DNA libraries to chromosome-specific libraries and in many cases now to site-specific probes. The approach of cloning DNA from chromosomal deletions should accelerate the tedious task of finding the gene responsible for a genetic disorder, as it greatly narrows down the set of sequences from which the gene has to be isolated.

Now, Monaco *et al.*³ describe how seven of these probes from the region deleted in one DMD patient were used to check for deletions in other DMD patients and also to do linkage analyses. Five of 57 males with DMD were found to have deletions, each missing at least 38 kilobases of genomic DNA. Two subclones detect restriction fragment length polymorphisms, which can be used to identify particular regions of DNA (for an explanation of this technique, see ref. 5). These subclones were used in a linkage study, showing that the clones represent DNA very closely linked to the DMD locus, so they can be used as a marker for the gene. It is interesting that nine per cent of the mutations at the DMD locus are deletions. In two other X-linked disorders, Lesch-Nyhan syndrome and ornithine transcarbamylase deficiency, deletions have been found in 18 and 7% of cases respectively^{6,7}.

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DMD is a genetic lethal because affected males do not live to reproduce. As the frequency of the gene will be twice as high in females as in males, one-third of the DMD genes are lost each generation. A similar number of new mutations arise to take their place. Thus, if male and female mutation rates are equal, one third of all DMD cases should be new mutations. There is quite a lot of controversy over this estimate but these probes should now enable us to resolve the dispute, as well as determine the relative mutation rates in the sexes. The most exciting aspect of these findings is that in the very near

future the DMD gene should be cloned and transgenic mice produced. These are major steps in determining the aetiology of DMD, which will in turn lead to fundamental insights into the basic mechanisms of this neuromuscular disease. □

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Immunology

Are peptides good antigens?

from G. Ada and J. J. Skehel

It is generally considered that most antibodies elicited after immunization with a protein in its native conformation will react well with the native molecule, but that only a minority will react with the denatured protein, with linear peptides derived from it or with synthetic peptide analogues. Initially, these conclusions came from studies (reviewed by M. J. Crumpton, ICRF) of the antigenicity of proteins such as myoglobin and of tobacco mosaic virus (TMV), in which it was also observed that antibodies to modified proteins on binding to native molecules caused changes in their conformation — for example, the binding of anti-apomyoglobin to myoglobin resulted in the release of the haem prosthetic group. Similarly, when protein fragments or synthetic peptides were used as immunogens, the resulting antibodies reacted poorly with the parent molecules. In the past few years the use of synthetic peptides as antigens has been extensively documented, and a Ciba Foundation Symposium was recently convened* to discuss advances in our understanding of the antigenicity of synthetic peptides and of their usefulness as biological reagents and as components of future vaccines.

Evidence in favour of the importance of segmental mobility of TMV protein in antibody recognition was reviewed by A. Klug (MRC, Cambridge). Seven continuous epitopes in the TMV protein have been located to regions in the molecule which correspond to sequences with high local mobility, as shown by X-ray crystallography. Such observations suggest that the local mobility of the polypeptide chain facilitate interaction of antibodies with the protein antigen, and that antibodies to these regions of high mobility react with peptide analogue because the peptides in solution can assume the conformation of the mobile segments. If, however,

peptides longer than eight residues are used to assign regions of antigenicity, additional epitopes may be found, corresponding to more structured regions of the molecule, including α -helices.

The molecular basis of the antigenicity of synthetic peptides was also considered by R. A. Lerner (Scripps Clinic) in a nuclear magnetic resonance study of the conformation of a peptide from influenza haemagglutinin. In solution the nine-residue peptide was found to contain a type II β -turn stabilized by a hydrogen bond between the carbonyl of the first residue and the amide group of the fourth, a conformation different from that which the peptide assumes in the native molecule. The possible antigenic significance of such a structure may be revealed by analysis of the structure of peptide-antibody complexes that are available.

As a contribution to the study of the degree of protein flexibility involved in antigen-antibody reactions, A. B. Edmundson (University of Utah) reported a study by X-ray crystallography of the interaction between a number of synthetic peptides and the binding site in an immunoglobulin light-chain dimer. *N*-formylated peptide antigens assumed shapes which conformed to the space available for binding in the main cavity of the site. The conformation of the binding site also changed to improve structural complementarity with the peptide ligand. Among the adjustments noted were rotations and translations of aromatic side-chains and expansion of the binding cavity by movement of the first, second and third hypervariable loops.

Several contributions dealt with practical aspects of the production of anti-peptide antibodies and peptide synthesis. PPD (purified protein derivative of tuberculin) reacts well with T cells but poorly with B cells. Coupling PPD to peptides enhances anti-peptide antibody production in animals pre-sensitized with BCG (P. J. Lachmann, MRC, Cambridge).

When inoculated into BCG-sensitized rats, PPD-peptide conjugates cause delayed-type hypersensitivity reactions which facilitate retention of the complex at the site of injection and possibly contribute to enhanced antibody production. M. Sela (Weizmann Institute) reported that covalent attachment of muramyl dipeptide to a synthetic poly-DL-alanine-diphtheria toxoid octapeptide conjugate greatly increases its immunogenicity. Immunity to cholera toxin can be elicited either by a polymerized peptide from the toxin or by peptide conjugated to a lipid moiety such as the dipalmityl lysine group. M. Geysen (Commonwealth Serum Laboratories) summarized a procedure for the rapid synthesis on a solid support of a large number of peptides and its application in a study of a putative discontinuous epitope of foot-and-mouth disease virus, the main antigen determinants of which are on the VP1 protein. A monoclonal antibody against the epitope which neutralizes virus infectivity but does not react with isolated VP1 polypeptides or with synthetic hexapeptides covering the complete VP1 amino-acid sequence was used as template. Peptides of increasing length were synthesized and assayed for antibody binding after addition of each residue, some of which were β -alanines to allow structural flexibility. Finally, an octapeptide that bound to the antibody to high titre was obtained.

Two papers were presented on the antigenicity of peptides corresponding to the repeat sequences which occur in many malaria antigens. V. Nussenzweig (New York University Medical Center) described studies of the species-specific circumsporozoite protein (CSP) in which a single epitope has been identified for all monoclonal antibodies. A dodecapeptide containing three four-residue repeats conjugated to diphtheria toxoid induces the formation of antibodies which react with both CSP and the immunizing peptide and which, in an *in vitro* system, is 90 per cent effective in preventing infection. Such preparations are expected to form the basis of an antimalarial vaccine. R. F. Anders (Walter and Eliza Hall Institute) reported on two antigens, S and RESA, isolated from the merozoite stage of *Plasmodium* infections. Peptides equivalent to portions of the repeat sequences of the S-antigen, which is released on schizont rupture, have been used to show that anti-S antibodies present in sera from infected individuals are specific for the repeat sequences. At present, such antibodies are not implicated in protection from malaria infection. RESA, the ring erythrocyte surface antigen, is inserted into the erythrocyte membrane at the time of merozoite invasion. It contains blocks of related but different repeat sequences; peptides equivalent to these repeats elicit cross-reacting and repeat-specific antibodies. The biological significance of these findings was discussed, particularly

* 'Synthetic Peptides as Antigens', London, 4-6 June 1983. The proceedings will be published under the title *Synthetic Peptides as Antigens* by Pitman Publishing, London, in February 1986.

in relation to the possibility that such cross-reacting antigens produced at different stages in the life cycle of the parasite may modify the host immune response to the parasite's advantage.

V.C. Stevens (Ohio State University) reported the use of a 37-amino-acid synthetic peptide corresponding to the carboxy-terminus of the human placental hormone, chorionic gonadotropin (hCG), as a component of a birth control vaccine. Since this sequence is not shared by pituitary hormones of similar structure, its use as an iso-immunogen is considered acceptable. The length of the peptide, which appears to contain type II β -turns in solution, is critical for optimum inhibition of biological activity. When conjugated to diphtheria toxoid and administered to female baboons in a squalene-water emulsion with muramyl dipeptide, the peptide prevents 95 per cent of pregnancies; in due course fertility is regained.

Two presentations provided examples of the use of anti-peptide antibodies as experimental reagents. In an analysis of the site on human IgG responsible for the production of auto-antibodies (rheumatoid factor), a peptide analogous to the glycosylated region in the $\gamma 1$ -domain has been found to be the most active in eliciting the autoimmune response (D.R. Stanworth, Birmingham University). Differences in the extent of glycosylation at this site may contribute to its autorecognition. Experiments were also described on the use of ϵ -chain peptides to elicit antibodies which might modulate IgE-mediated hypersensitivity in rats and humans. G. Evan (Ludwig Institute, Cambridge) described the use of peptides to produce monoclonal and polyclonal anti-

bodies against the product of the *myc* gene and their use to identify the intact *c-myc* phosphoprotein in normal and transformed cells. They have also been used to localize the protein in cell nuclei in culture and in tumour masses by whole-body detection of ^{125}I -labelled antibodies.

Considerable enthusiasm has been generated for the use of peptides as components of vaccines, and the results obtained with CG and malarial CSP peptide analogues have gone some way to support this. In both cases, the size of the immunizing peptide is important and this may indicate a requirement for more than one sequence in a peptide to be recognized, or that peptides of these sizes more readily assume conformations required for immunogenicity. But it also became clear that many synthetic peptides do not induce antibodies that react with the native parent protein, arguing against the general applicability of peptides in vaccine production at this stage. Such observations also have implications for the interpretation of basic studies of protein antigenicity in which the definition of antigenicity is simply based on the characteristics of the assay systems employed, which often involve the use of peptides or modified proteins as antigens. As a consequence the criteria which have been singly deduced from these studies to be indicative of antigenic regions in native proteins are unlikely to be universally applicable. $\square$

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Computers

More parallel than others

from D. Parkinson

PARALLELISM in computers takes many forms. Single operations use parallelism to enhance performance by, for example, fetching all 'bits' of a word simultaneously from memory or by using parallel circuitry to speed up operations such as addition or subtraction. Single-operation (bit-parallel) has been common since the earliest computers, when manufacturers began to use 'pipelined' operations to speed up the execution of streams of instructions. Pipelining is the computer's equivalent of the industrial production line, in which a complex operation is split into several sub-tasks and the operands are fed through a series of specialized facilities that perform these sub-tasks. It is a well-established procedure in computer design and usage, but the invention of the vector processor and creation of general purpose multiple-processor systems have awakened new interest in the possibilities

of parallel processing.

As a trivial example, imagine the following basic operations on a stream of instructions: fetch the instruction from memory; decode the instruction; obey the instruction. A three-stage pipeline could be constructed that would allow all three instructions to be analysed simultaneously. The first would be obeyed while the next is being decoded and the third is being fetched, the process being three times faster than a non-pipelined system.

The pipelining principle is also well-established for building high-performance signal or process-control systems. Here the tasks in the pipeline might be allocated to separate special-purpose boxes for performing operations such as calibration and convolution or transforms. Both these applications of pipelining are examples of parallelism, but they display parallelism at different levels of 'granularity'. The

instruction-decoding example is fine-grain parallelism where the amount of work between synchronization activities is small. The signal processing application exploits coarse-grain parallelism where large amounts of computation are carried out between synchronizations. There is, of course, nothing that prevents the operations of the coarse-grain system being implemented by systems that exploit fine-grain parallelism.

The vector processor, such as CRAY-1, CYBER-205, is a further refinement of the pipeline principle to perform repeated application of identical instructions to sets of data, the so-called vectors. If we wished to perform the operation $x(i) + z(i)$ ($i = 1 \dots N$), then increased performance is possible if the hardware can perform the instruction-decode operation only once and include all indexing operations in the pipeline. Vector processors have special facilities to perform these operations and so can provide increased performance for that range of computations which can be expressed in terms of the vector operations. The instruction-decoding base for a vector instruction is more complex than for a simple scalar instruction, so there must be minimum number of elements in the vectors before vector instructions can be faster than a set of scalar instructions. This characteristic of vector processors means that the programming or computational methods for full exploitation of a vector processor can be very different from those used in the simple early computers.

Alongside the development of vector processors, multiple-processor systems have been developed consisting of many interconnected computers, all of which can be used to solve a given task. The earliest system was the ILLIAC IV, intended to be a 256-processor system but only one quarter of the machine was ever built. Although great computational power could be achieved on parts of problems, many problems suffered large-scale degradations because of inadequate facilities for moving data amongst the processors.

The attractions of obtaining increased performance by using many computers is obvious, hence the many attempts to develop such systems. To date, the most successful of these have been two designs in the United Kingdom — the DAP from International Computers Ltd and the CLIP IV from University College, London. Six DAPs have been installed since 1980, each with 4,096 processors in a 64×64 array. They are used in a wide range of general-purpose computations. The CLIP is available commercially in various configurations of 32×32 , 32×64 and 64×64 processors.

The processor arrays are specialized in the sense that they all respond to a single instruction stream broadcast from a master processor. Hence, if one processor is performing an addition operation, all the

other processors also perform an addition operation (which might be a dummy). Although this 'lock-step' characteristic appears very restrictive, most user experience has shown that such lock-step processor arrays are highly flexible. Processor arrays and vector processors are often called single instruction multiple datastream (SIMD) processors, as a single instruction produces many results.

It is generally accepted that a collection of processors that did not have to work in lock-step fashion would be more flexible than a SIMD system. Therefore, there are many attempts to develop multiple instruction datastream (MIMD) systems, mainly as part of programmes such as fifth generation ALVEY and ESPRIT. One of the dreams of computer scientists is the ideal MIMD computer with the following properties: it supports a programming methodology that does not need to know how many computers are in the system; it can double in performance if the number

of computers is doubled; and it automatically rejects any failing computer yet continues to work.

The production of computers with these desirable properties is a major research target. Nearly every day there is a new proposal for a computer architecture system which its designers claim will meet the aims. Many one-off experimental systems exist in universities, but none seem to have gained external acceptance. There is, perhaps, an analogy between the programmes to build an ideal MIMD system and that to build an operational fusion power generation plant. Everyone agrees that it would be a good thing to do, lots of money is being thrown at the problem, but nobody has yet shown that it can be done. However, interesting things are happening—watch this space! □

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Quantum fluid dynamics

Instabilities at absolute zero

from K. W. Schwarz

ILLUSTRATIONS of flow around a sphere are a seemingly essential part of every textbook on fluid dynamics. At low velocities, the fluid is seen to make its way smoothly past the sphere. The nonlinear nature of flow phenomena, however, becomes spectacularly evident at higher velocities, with the appearance of a vortex ring (similar to a smoke ring) in the wake of the sphere. At still higher velocities, the ring is intermittently shed into the fluid, leading eventually to completely turbulent flow behind the sphere. McClintock, Bowley and colleagues, on page 797 of this issue¹, examine the quantum mechanical analogue of this venerable problem. In the latest in a series of remarkably elegant experiments, using electrons injected into liquid helium to form bubbles, they find, unexpectedly, that when a bubble is pulled very quickly through the fluid a stream of microscopic quantized vortex rings is generated. The similarity to classical flow around a sphere is both striking and aesthetically satisfying, and it should help us understand better the appearance of instabilities in quantum fluids, such as ⁴He.

Helium-4 remains liquid down to absolute zero. In this limit, it obeys the laws of quantum mechanics, which operate on a macroscopic scale under such conditions. The rules governing the motion of this 'superfluid' are therefore very different from those of classical fluid dynamics, and are still only imperfectly understood. Two kinds of excitations are known to be possible in superfluid ⁴He: vibrational excitations, somewhat analogous to sound waves, and flow excitations in the form of quantized vortex lines. The latter are

essentially localized microscopic whirlpools, the fluid circulating rapidly around a central line about 0.1 nm thick, with the velocity dropping off away from the core. Such quantized vortex lines can exist as arbitrarily configured continuous curves in the fluid, either as segments which terminate on boundaries, or as free-floating loops and rings. Once there, they obey the laws of classical fluid dynamics. What is not understood is how they arise in the first place as an instability of quantum flow. Thus it is of the utmost interest to study how 'superflow' past a simple object such as a sphere becomes unstable.

To carry out their experiments the authors make use of the well-established fact that an excess electron injected into liquid helium forms a well-defined spherical bubble with a radius of about 1.5 nm. Such electron bubbles can be accelerated through the superfluid by applying an electric field, and their motion can be detected as a current. Since the movement of such a structure displaces thousands of helium atoms, the flow around it can be thought of as macroscopic. Yet, the dimensions of the bubbles are so small that any quantum instability that occurs can be expected to involve individual excitations of the kind described above. Earlier investigations^{2,3} had in fact established that, depending on the pressure, a rapidly moving electron bubble will either create a single vortex ring to which it remains attached, or it will emit a continuous stream of vibrational excitations. Thus, depending on circumstances, instability with respect to either type of possible excitation was known to occur.

Although they have also made major contributions to our knowledge of the vibrational-excitation instability⁴, McClintock, Bowley and coworkers have more recently concentrated on the situation where the flow around the bubble becomes unstable leading to the creation of quantized vortex rings, a situation that appears to more closely resemble the classical problem. By doing experiments in isotopically pure ⁴He at sufficiently low temperatures, the scattering of thermal vibrations by the moving bubble becomes rare, so they have been able to study the problem in its purest form. That is, they can look at what happens as the bubble is pulled more and more forcefully through the zero-temperature superfluid. They find that initially the bubble accelerates exactly as would a sphere moving through an ideally frictionless classical fluid⁵. When a critical velocity is reached, a quantized vortex ring attached to the sphere is created. The unexpected result of their latest experiment¹ is that, when a very large field is applied to pull the bubble through the fluid very quickly, a continuous stream of vortex rings is emitted by the moving bubble.

These latest results promise to lead to new insights into the nature of quantized-vortex creation. At present, not much is understood theoretically about this process. An early calculation by Schwarz and Jang⁶ demonstrated that the critical velocity is determined by the instantaneous application of energy and momentum conservation, implying that the vortex appears as the result of a sudden quantum transition. This point of view appears to be generally accepted today and much more ambitious models have recently been proposed^{7,8}. The subject, however, is controversial, and in particular the very basic issue of defining the configuration in which the quantized vortex first appears remains under debate. By comparing experimental results of the kind reported here against hydrodynamic calculations using various starting geometries for the vortices, it may now be possible to determine this initial configuration. Prospects for attaining this milestone seem encouraging, although a great deal more theoretical and experimental effort will be required. □

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Cytoskeleton

Splitting hairs and other intermediate filaments

from Peter M. Steinert and Alasdair C. Steven

EVEN though keratinized tissues rich in intermediate filaments were possibly the first biological substances to be probed by X-ray diffraction techniques (ref. 1), solving their structure remains a plum to be picked. These filaments, 10–15 nm in diameter, form one of the three major cytoskeletal networks in eukaryotic cells. They differ from the other two, F-actin and microtubules, in several important respects. For example, unlike the other two, intermediate filaments are composed of α -helix rich fibrous protein subunits, and their distributions and state of assembly in cells seem to be governed by quite different rules. Their precise function(s) in cells remain obscure, so an understanding of the details of their structure is an important first step in elucidating their cellular functions. A flurry of recent papers describing the amino-acid sequences of around 30 subunits suggests that the broad principles of intermediate filament structure will soon be understood.

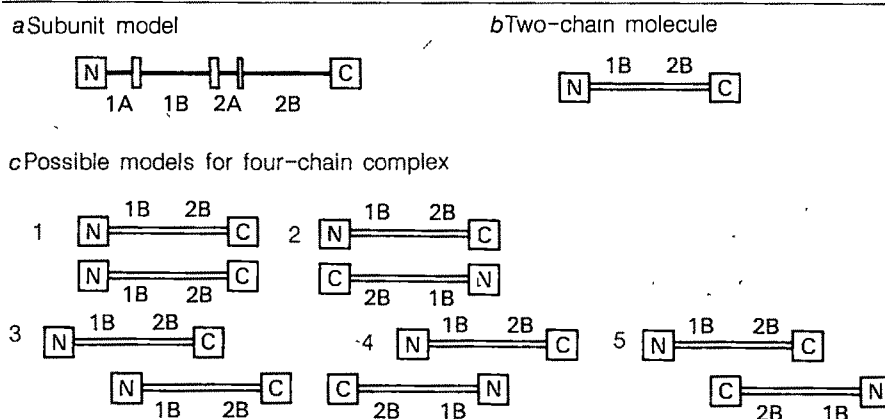
All the filaments have been found to contain a central α -helical 'rod' domain of conserved secondary structure, flanked by end domains of variable size and chemical character. The rod domains possess four discrete segments of precisely conserved size that can form coiled-coils, termed 1A, 1B, 2A and 2B, interspersed by regions that cannot form coiled-coils (*a* in figure). The rod domain sequences conform to several distinct types: the 10–15 acidic keratins (Type I); the 10–15 neutral-basic keratins (Type II); vimentin, desmin and glial fibrillary acidic protein (Type III); and neurofilament proteins (possibly comprising at least one other type²). Convincing evidence now shows that pairs of subunits align in parallel and in axial register to form two-chain coiled-coil molecules (*b* in figure)² but it is not known how these molecules combine to generate higher orders of intermediate filament structure. Observations using the scanning transmission electron microscope³ suggest that most filaments,

irrespective of their subunit composition, contain about 16 molecules in cross-section. These and other data have also contributed to the emerging concept that the rod domains form the cores of intermediate filaments and that the interactions of these domains are primarily responsible for defining filament structure. The end domains are located on the periphery of this core and define the functions of different filaments in cells^{2,3}.

At the immediately supramolecular level, several lines of evidence invoke a dimeric association of molecules in a four-chain oligomeric complex^{4,5}. It is plausible that such an association should be based

particles, but interpreted them not as models 3–5, but as staggered pairs of four-chain complexes. Again, the complexes might be parallel or antiparallel, but their neighbouring constituent two-chain molecules should nevertheless comply with models shown in *c*.

The ingenuity of these experiments notwithstanding, their apparent incompatibility poses a problem: which data are correct? Or is every investigator right? Assuming that keratin and Type III intermediate filaments do share a common structure, there may be at least one way to reconcile this paradox. Conceivably, there may be multiple modes of intermolecular associations in fully assembled filaments, so that more than one of the models in *c* may be applicable. Then, disassembly or reassembly of intermediate filaments under conditions of varying salt or pH might selectively weaken different sets of interactions and split the same structure into different four-chain complexes. This idea could be tested by the



on alignment of the long (1B and 2B) coiled-coil segments²; if so, there are essentially only five possibilities (*c* in figure). Models 1 and 2 predict the segments to be about 48 nm long and the others predict them to be about 70 nm.

Two recent studies have cast some light on these alternatives. Using quick-freeze deep-etching electron microscope techniques, Ip *et al.*⁶ have characterized a 48 nm particle as the tetrameric building block for vimentin. This is consistent with models 1 or 2, but cannot distinguish between them as the differences depend on the polarity of the molecules. But, by using a monoclonal antibody against region 2B of the desmin rod, Geisler *et al.*⁷ seem to have answered this question. They found dumbbell-shaped structures averaging 48 nm in length, denoting antibody binding at both ends of a similar tetrameric desmin complex and compatible only with model 2. However, in other studies on isolated α -helix-enriched particles obtained from proteolysed keratin intermediate filaments, 1B tetramer particles were obtained^{8,9} which could only have derived from models 1 or 4 and not from model 2. Under modified salt conditions, Ip *et al.*⁶ visualized larger 70-nm-long vimentin

experimental approaches described here.

Taken together, recent reports make it likely that neighbouring pairs of two-chain coiled-coil molecules in intermediate filaments are antiparallel, meaning that the filaments are non-polar structures. Thus, unlike the other mainstays of the cytoskeleton, F-actin and microtubules, intermediate filaments don't know whether they are coming or going. Further developments in this rapidly evolving field hopefully will extricate researchers from a similar dilemma. □

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Peter M. Steinert is in the Dermatology Branch, NCI, and Alasdair C. Steven is in the Laboratory of Cellular and Developmental Biology, NIADDK, National Institutes of Health, Bethesda, Maryland 20205, USA.

100 Years Ago

MANY interesting excursions have been arranged for the Aberdeen meeting of the British Association. One of them will, of course, be to the great granite quarries in the neighbourhood of Aberdeen. Her Majesty has invited 150 of the members to Balmoral, where they will be shown over the grounds and have lunch. It is not to be expected that the Queen will personally receive all the members, though it is possible that a few representative men of science may be presented to Her Majesty.

From *Nature* 32 400, 27 August 1885

Plate tectonics

Room at the top of the world

from J. Tuzo Wilson

BENEATH the ice the Arctic Basin is vast, poorly known and difficult to study. Perhaps the only thing that can be said with certainty is that its geology and geophysics are complex. This lack of information has enabled some scientists to claim that there is no room in the Arctic for the global motions of the Earth's crust required by plate tectonic theory. This raises a dilemma for supporters of that theory, but fortunately a broader view and new data from two expeditions (Weber, J. R. & Sweeney, J. F. *J. geophys. Res.* **90**, 663, 1985; Sweeney, J. R. *Geos* **13**, 8, 1984) suggest alternative solutions.

One aspect of the problem is that most mid-ocean ridges (where new oceanic crust is created) are aligned roughly north-south, spreading out from a ring through the southern oceans (Fig. 1a). This rough symmetry of the mid-ocean ridge system is a relic of the break-up of Gondwana which commenced about 250 Myr ago (Fig. 1b). At that time, the southern continents were clustered round Antarctica. But since the girth of the globe increases towards the Equator, the continents were forced to separate as they moved slowly northwards. This spreading apart of those continents which once surrounded Antarctica like blocks in an arch explains why they all have triangular or trapezoidal shapes narrowing to the south. Once across the Equator, one would expect the continents to be forced together again and to override mid-ocean ridges, just as North America has overridden the East Pacific Rise. If forced to converge in Arctic regions, there would not be enough space

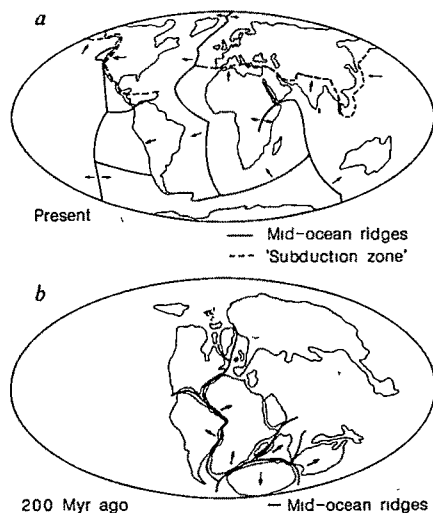


Fig. 1 Cartoon maps of the world as it is now (a) and as it was 200 Myr ago, before the break-up of Gondwana (b). The spreading centre which formed a ring around Antarctica has expanded greatly, driving the continents northwards. Today, a subduction zone decouples the movements of the southern and northern continents.

for them or to permit any fresh spreading.

As if not content with the rate of progress towards such a continental crash, dozens of fragments like those of Baja and western California have been moving polewards up the coast of North America. This has created a complex of terranes in Alaska and British Columbia, which led P. B. Jones (*Can. Soc. Petrol. Geol.* **80**, 83, 1982) to suggest that all of Alaska is moving towards the Arctic Basin. But that view is at odds with new data emerging from Canadian expeditions which indicate extension rather than compression in the Arctic. The impasse can be avoided if account is taken of the globe-encircling zone of subduction and continental collision which separates the Gondwana continents from the others. This zone extends from the Mid-Atlantic Ridge near the Azores eastwards along the active mountain and island arcs of southern Eurasia to Indonesia and thence around the rim of the North Pacific Ocean to Central America and the Caribbean (Fig. 1a). The sliver of western California which is now moving north lies on the southern side of this zone. This suggests that rather than pushing into the Arctic Basin, the fragments now forming Alaska could have been accreted at a subduction zone which 'insulated' the Arctic.

An important question in plate tectonics is whether ocean basins are passively pulled apart at mid-ocean ridges, or whether the spreading is driven actively by forces originating in the mantle. D. I. Gough (*Nature* **311**, 428, 1984) has shown that a whole variety of phenomena, ranging from topography to *in situ* stress measurements indicate that active spreading is continuing beneath the western United States in the region which has overridden the East Pacific Rise. This supports the latter possibility, which is lent further credence from recent discoveries in the Arctic Basin.

The chief conclusion from the LOREX (Lomonosov Ridge Expedition) expedition, reported by Weber and Sweeney, is that the Lomonosov Ridge (Fig. 2) is a remarkably uniform, steep-sided, flat-topped feature with at least one kilometre of flat-lying sedimentary layers underlain by a root, extending perhaps to 25 km depth within which the density increases downwards from 2.5 to 2.95 g cm⁻³. This lends support to the widely accepted view that the ridge is a block-faulted fragment of continental margin detached from Siberia 53 Myr ago by normal seafloor spreading at the Nansen-Gakkel Ridge in the Nansen Basin.

Weber and Sweeney suggest from limited data that the Alpha Ridge may once

have been joined to the Lomonosov Ridge and have had a similar origin, but that extension, including faulting, and injection of much mafic rock (as in the Lord Howe Rise north-east of New Zealand) can explain its thinner crust and magnetic lineations. They also admit that it could be a plateau of thick oceanic crust. Other results indicate that the Canadian end of the Makarov Basin is a faulted trough 25–60 km wide produced by extension "other than typical seafloor-spreading". It is partly filled with flat-lying sediments and has a crust 14 km thick.

Preliminary analysis of data gathered on a later expedition — CESAR (Canadian Expedition to Study the Alpha Rise) — reported by Sweeney, strengthens the idea that the Canada Basin also opened by



Fig. 2. Sea floor geography beneath the North Pole, showing the main tectonic features.

spreading when a crustal block, comprising northern Alaska and northwestern Siberia, which had lain against the Canadian Arctic Islands, rotated to its present position. Imprecise age determinations suggest that continental break-up began some time between 131 and 113 Myr ago and that new sea floor was formed from 118 to 79 Myr ago. H. R. Jackson, D. Forsyth, & G. L. Johnson (personal communication) support these views and show that the thickness and seismic velocities beneath the Alpha Ridge correspond to those of the thickened oceanic crust beneath other large oceanic plateaus, including the Manihiki, Shatsky and Hess rises. All these features formed during the magnetically quiet period in the late Cretaceous, probably at complex plate junctions.

The inescapable conclusion to be drawn from these studies is that despite the expectation from global tectonics that the region would be under compression, abundant spreading has in fact occurred. Together with the evidence for active spreading under the western United States, the new data from the North Pole support the view that spreading is caused by powerful forces within the mantle. □

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ALREADY
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The fall of Nikolai Vavilov

J.R.S. Fincham

The Vavilov Affair. By Mark Popovsky. *Archon/Clio:1985**. Pp.216. \$22.50, £21.40.

This book, by a former Soviet writer now resident in the United States, traces the career, downfall and destruction of the most famous Soviet biologist of the inter-war period. N.I. Vavilov was the son of a wealthy self-made businessman and one of two brothers, the other a physicist, who both became Academicians.

As a young man Vavilov spent two years (1912–1914) in England under the tutelage of the geneticist William Bateson and the plant breeder Rowland Biffen. At the time of the 1917 revolution, when he was 30, he had just been appointed to a professorial chair in Saratov. In 1920 he left Saratov to become head of the Bureau of Applied Biology in Petrograd and five years later was appointed Director of the new All-Union Institute of Applied Biology (in effect, Plant Breeding), the foundation of which, according to Popovsky, was a consequence of Lenin's having read an American book on the great potential of plant breeding. This Institute became the headquarters, and Vavilov the chief, of a great network of research institutes and experimental stations employing, it is said, as many as 20,000 people. With these resources at his disposal, Vavilov devoted his great energies to organizing and leading plant-hunting expeditions to many parts of the world and established very extensive collections of wild relatives of cultivated plants for investigation in his own institutes. He also wrote indefatigably on plant geography, the history of agriculture and origins of cultivated plants, and the theory and practice of plant breeding, with emphasis on the use of wild species as sources of such valuable traits as disease resistance.

From the early 1930s, Vavilov's enterprises came under increasing challenge from the ambitious T.D. Lysenko whose position, which may be summed up as Lamarckist, anti-élitist and ignorant, attracted increasing political support. In the Soviet climate of the time, criticism of Vavilov's science as academic and insufficiently practical slipped easily into accusations of philosophical and ideological error and eventually of treason. Vavilov was arrested while on a collecting expedition in August 1940, and sentenced to death as a saboteur and spy in July 1941, but was not in fact executed; he died in the prison hospital in Saratov in January 1943.

Mark Popovsky started his research into Vavilov's life in 1964. At that time the process of posthumous rehabilitation was already well under way, and Popovsky was able to gain access to a number of different sources. For the period up to 1940 he cites mainly the archives of the Lenin Academy of Agricultural Sciences (from the Presidency of which Vavilov was displaced by Lysenko in 1933) and of Vavilov's old Institute. He also interviewed a considerable number of Vavilov's former colleagues. In 1966 he published a narrative based on this information under the title 'A Thousand Days of Academician Vavilov' in a Soviet magazine. This account was cited (with some critical amendment) by Zhores Medvedev in his definitive book *The Rise and Fall of T.D. Lysenko*, published in I.M. Lerner's English translation in 1969.

The most novel part of the present book is based on the massive police files on Vavilov to which Popovsky was rather amazingly granted access. These contained a full record of Vavilov's lengthy interrogation and brief trial, as well as a large collection of much earlier material — letters from Vavilov's opponents and from police informers — and some later letters from Vavilov appealing for clemency. Popovsky describes his daily visits to the procurator general's office in Moscow, where he sat making notes under the eye of an invigilator who allowed him to see more than he was supposed to, assuming, Popovsky conjectures, that a person of such affrontery must be acting on higher

authority. The official purpose of this exercise is not made clear. There was obviously no intention of allowing Popovsky to publicize his findings. Indeed, he subsequently ran into increasingly bad trouble with the authorities for disclosures. In 1977 he was allowed to emigrate, and managed to smuggle some of his notes out with him, much of the rest having already been sent abroad.

So what do we learn from this new evidence? We obtain some insight into the material — a mixture of bigotry and personal malice — from which the charges against Vavilov were fabricated. We also learn, not too surprisingly, that, under pressure of prolonged interrogation, Vavilov was prepared to compromise. He admitted the charges of deliberately leading Soviet plant research along wrong paths and was prepared to incriminate others (already dead) as accomplices in that crime. However, he continued to deny that he had been guilty of espionage. He never lost hope that, even though imprisoned, he would be allowed to resume scientific work, perhaps in the kind of institute of captive scientists described in Solzhenitsyn's *The First Circle*. It appears that in June 1942 Beria had decided to permit this, but that in the confusion of the war the plan was forgotten or countermanded. At all events, seven months later the opportunity was lost and Vavilov was dead.

In the final chapter of his book Popovsky recounts what he was able to find out as a member of a group sent to Saratov by a Commission of Investigation set up by the Soviet Academy of Sciences. This group interviewed, not very fruitfully it appears, some of the prison hospital staff who had charge of Vavilov during his last days. Popovsky devotes several pages to the cause of death, accusing the prison staff of falsely misrepresenting it as pneumonia



Before the storm — Vavilov in 1928, driving an electric plough on a station belonging to the Central Asian State University. With him is his son Oleg.

*Archon, Box 4327, 995 Sherman Avenue, Hamden, Connecticut 06514, USA. Clio Distribution Services, 55 St Thomas' Street, Oxford OX1 1JG, UK.

when it was really starvation. It hardly seems to matter much, and one supposes that it may well have been a combination of the two; the circumstances must have been conducive to both conditions.

What was Vavilov's standing as a scientist and what did he achieve? In his own time he enjoyed a considerable international reputation, demonstrated by his election as President of the 1939 (Edinburgh) International Congress of Genetics (which he was prevented from attending). This was no doubt in part a gesture of support for a fellow scientist in difficulties, but was also a recognition of his scientific stature, recognition that was reaffirmed in a special session of the 1980 (Moscow) Congress. But, tragically, much of his work was aborted. His plant collections were largely lost and most of the great books that he had planned could never be written. His most important theoretical contributions, the idea of centres of origin of cultivated plants and the Law of Homologous Variation (which may be para-

phrased by saying that related species have similar genes subject to similar kinds of mutation), hardly bulk large in today's textbooks; they have achieved the status of the valid but obvious — the fate of many good ideas in science. Vavilov's commitment to the preservation and exploitation of wild species is now widely shared, and this may well be his most important scientific legacy. But he will probably be most remembered as a victim of the perversion of science in the service of ideology and the struggle for power.

Mark Popovsky has written a most saddening and thought-provoking book. As a work of history it suffers from the defect that its most important sources cannot be checked, either by the reader or now by the author himself. But it is as good a biography of Vavilov as we are likely to get in present circumstances. □

J.R.S. Fincham is Arthur Balfour Professor in the Department of Genetics, University of Cambridge, Downing Street, Cambridge CB2 3EH, UK.

Sweet — and sour

John Yudkin

Sweetness and Power: The Place of Sugar in Modern History. By Sidney W. Mintz. Viking: 1985. Pp.274. \$20, £14.95.

DURING the past three centuries, the quantity of sugar consumed in Britain has risen from an average per person of 4 lb a year to the present 100 lb, so that it now constitutes a greater proportion of the diet than does any other food, or indeed a greater proportion than that contributed by all the meat, poultry, eggs and fish that we eat. There have been similar increases in sugar consumption in other industrialized countries, although they began more recently. By itself, sugar would not be consumed in very great quantities, and then only in solution. But in combination its wide range of properties make it suitable for use in sweetmeats and fruit preservation, for enhancing the taste of vegetables and meats, for making jam and ice cream, and for making intricately sculptured designs ranging from the icing on cakes to large sculptures of figures and buildings.

The cultivation of sugar cane began in the Far East about 500 bc and during the seventeenth and eighteenth centuries in particular spread rapidly to many parts of the world where the climate is tropical or sub-tropical. Because it needs intensive labour, the growing of cane as a crop resulted in a massive movement of populations and was responsible for the resettling of great numbers of Africans in the Caribbean and central and north America, and for the extinction of the indigenous population of several of the Caribbean islands. It is difficult to exaggerate the demographic, cultural, social and economic effects of the importation of black slaves to the Western hemisphere. Among other things, it was responsible for the dependence of the sugar-growing countries on what virtually became a monoculture, and thus for the distortion of local agriculture, local food supplies and local patterns of nutritional deficiencies.

What was the cause of the enormous increase of sugar growing in these countries and its consumption both there and in the industrial nations, beginning with Britain? In his wide-ranging account of the history of the subject, Dr Mintz mentions such factors as rapid developments in the efficiency of production of the cane and its yield of sugar, technical improvements in refining, the introduction of sugar from beet, the spread of the drinking of hot beverages — tea, coffee and chocolate — as well as the discovery of eating chocolate, and, finally, the rise in the purchasing power of individuals in the consumer nations. He also convincingly develops the theory that production and

Something in the air

James E. Lovelock

Chemistry of Atmospheres: An Introduction to the Chemistry of the Atmospheres of Earth, the Planets, and their Satellites. By Richard P. Wayne. Clarendon: 1985. Pp.361. Hbk £30, \$39.95; pbk £14.95, \$19.95.

TEXTBOOKS about the atmosphere have until now not fully reflected the new knowledge from space exploration. Just as important, they have failed to observe that the atmosphere is essentially a biological product. These earlier texts stand in science rather as does the Old Testament to a Christian; not wrong, merely unfulfilled. Richard Wayne's admirable book is definitely of the New Testament of atmospheric science.

The book is easy and enjoyable to read. It is a fine piece of craftsmanship, with a most comprehensive bibliography and index, and illustrates with clarity the interactions between the professional practice of physical chemistry and the larger domains of science and the world itself. It is especially refreshing to find illustrations taken where appropriate from sciences ranging from astronomy to zoology. Here the demarcation disputes over the territorial boundaries of science no longer imprison our imagination.

My only point of difference is over the space given and the deference shown towards that curious obsession of aeronomists — impending doom from the depletion of stratospheric ozone. We inhabit a world where the environment is threatened by carbon dioxide increase, changes in land use, acid deposition and a rise in the concentration of tropospheric

ozone. To spend more than a few words on the lesser ailment of stratospheric ozone depletion seems out of balance. I well understand why physical chemists are transported by thoughts about the stratosphere and the ozone there. Ozone is a key constituent of an important part of the atmosphere and the stratosphere is an near-ideal reactor for the study of photochemical reactions, no less than 250 of which have so far been listed. It should not be necessary to use doubtful arguments about a possible increase of skin cancer in the next century to justify such important research. This disagreement over emphasis in no way detracted from my enjoyment of the thorough discussion in the book of the intricate chemistry of real or potential pollution problems. There is also proper and decent speculation about consequences and on what more we need to know.

Recent explorations have brought the upper atmosphere down to Earth. The chapters on ionization and on the air glow nicely bring together the old and the new chemistry of this upper region of the air. And the account of extraterrestrial atmospheres describes the very different chemistry of those barren worlds and provides a contrast to that of the Earth where life has created a strange and beautiful anomaly in the Solar System.

Chemistry of Atmospheres is much more than a first-rate student text. It is also a scholarly interim report on the state of the subject as seen by a broad-minded physical chemist. Anyone with a serious interest in the atmosphere and the chemistry of the gaseous particles it carries will benefit greatly from reading Richard Wayne's book. □

James E. Lovelock is an independent scientist working at Coombe Hill, St Giles on the Heath, Launceston, Cornwall PL15 9RY, UK.

distribution of sugar powerfully affected the relationship between the wealthy and the labouring classes: "Probably no single food commodity on the world market has been subjected to so much politicking as sugar". This is a view that, in more lurid detail, is exposed by Al Imfeld in his recent book *Zucker* (unfortunately not yet translated from the German). Together with *Sweetness and Power*, these accounts serve as an illustration of the extent to which the study of this universally popular food is the province of the historian, economist, demographer, geographer, anthropologist and sociologist, as well as the clinician and scientist.

As a final attractive titbit, Dr Mintz



Enough to make you sick? Etienne Tholoni, a great French sugar baker, puts the finishing touches to a life-size chocolate nude with spun-sugar hair. She is lying on a bed of six-hundred sugar roses. (Centre de Documentation du Sucre; reproduced from *Sweetness and Power*.)

serves up a splendid dessert in which he discusses the current changes in the traditional eating patterns of industrialized societies, and the extent to which the multifarious uses of sugar play a part. The wide and increased proportion of specialized manufactured food and snacks, and the fast-food revolution, have changed when, where, what, how much and how quickly people eat, and the result is to produce what Dr Mintz calls "desocialized eating".

This is a book that can be read for its fascinating story or studied as a well-researched, scholarly and excellently documented work; it is beautifully written, with a good index, copious bibliography and extensive notes. Such a combination of qualities is a rare commodity — unlike the ubiquitous substance that is the subject of the book. □

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Eating for survival in China

Brian Bertram

The Giant Pandas of Wolong. By George B. Schaller, Hu Jinchu, Pan Wenshi and Zhu Jing. University of Chicago Press: 1985. Pp.298. \$25, £23.75.

WE HAVE been waiting for years for data on giant pandas in the wild in China. A full-scale collaborative research programme in the field started there at the end of 1980; it is still continuing, but the results of the first 18 months of work have now been published.

George Schaller is renowned for his ability to obtain superb data from large wild mammals — gorillas, lions, snow leopards — under difficult conditions. With his work on giant pandas the tradition continues. As the report makes clear without emphasizing the point, pandas are exceedingly difficult creatures to study. Quite apart from the administrative, logistical and language problems involved, the animals inhabit dense bamboo forest on steep mountainsides. The climate is unlovely: "... cool and wet ... snow fell between late October and April ... on rare days when the rainclouds part and sun reaches the valley ... even when it did not rain, fog continued to swirl along slopes, everything remained sodden ...". And the quiet, shy and relatively inactive pandas cannot be seen through the bamboo if more than 10–15 metres away, and they do their best to remain further away than that. During a 16-month preparatory phase, the research team saw pandas only 39 times. Only radio-tracking makes them at all possible to study, but still not at all easy.

The team had radio-collars on six individuals, and so managed to get information from over 1,500 panda locations. In the three main data chapters they present the results. The hard-earned information is fascinating, but pandas basically are not exciting for the very good reason that their lives are largely taken up with eating bamboo (over 99 per cent of their diet). Nonetheless, the book brings out beautifully the complex and highly unusual interaction between the panda and its food supply.

Giant pandas, as carnivores in origin, have a short gut in relation to their body size and to their food needs, and they cannot break down cellulose. Digestion is inefficient, and the food passes through the gut rapidly. A 90 kg panda has to take in between 12 and 38 kg of bamboo per day; the amounts vary with the season, and depend on whether the panda is feeding on leaves (in summer), leaves and young stems (in winter) or old stems and new shoots (in spring). It defaecates about 48 times per day, the faeces totalling 20 kg

or more in weight. The nutrient level of bamboo apparently remains relatively constant through the year, but while bamboo is always abundant it is time-consuming to gather. Protein intake levels seem adequate, and the prodigious amounts consumed are probably required to obtain enough digestible carbohydrate, particularly in spring, the lean season for pandas. At this time of the year, the limitation on the animals' intake of food may be their guts' ability to deal with the bulk.

A panda in the Wolong study area spends most of its life taking in food. It forages for 13 hours a day, rests (conserving scarce energy) for 10 hours, and fits everything else (which is not much — drinking, travelling, grooming, defaecating, scent-marking, playing and socializing) into the one remaining hour. It is no wonder therefore that the home ranges and the daily distances travelled are both small, and the animals very largely solitary.

One chapter brings together the information on reproduction, breeding behaviour and communication from captive studies in zoos and from this first major field study. There is probably delayed implantation; this is suggested by the extraordinarily variable gestation period (ranging between 97 and 163 days in fewer than 30 measured pregnancies in captivity), the time lag after copulation before a progesterone rise, and the minute size (90–130 g) of the one or two offspring at birth. At about 0.011 per cent of maternal weight, these are the smallest eutherian mammal babies known.

Anatomical, physiological and behavioural features are used to re-examine the old problem of giant panda phylogeny. The species appears in many independent respects to be quite closely related to the bears, whereas the much smaller red panda seems to be close to the Procyonids (the raccoon and its relatives). But in many other respects giant pandas seem to be merely enlarged red pandas and phylogenetically close to them. There has clearly been a much greater degree of convergence in evolution than is comfortable to taxonomists. The authors' solution to the contradictions is the more usual one of considering red and giant pandas to be the closest relatives, and therefore of necessity distancing both from both bears and raccoons. This is not the conclusion of the most recent molecular studies in which O'Brien *et al.* (*Nature*, in press) have asserted that the giant panda is close to the bears and the red panda close to nothing else.

The data on giant pandas in the wild will be useful in managing the small wild population, estimated at about 1,100, mostly in a few circumscribed reserves. But equally valuable will be the fact that the study has focused international interest on the plight of the giant panda and on the steps which will need to be taken

for the conservation of the species and its habitat.

The long-cycle flowering and subsequent temporary die-off of bamboo, and consequent starvation of pandas, has received a great deal of publicity. The situation is very complex. In "proper" panda habitat, with a variety of bamboo species, each flowering intermittently but asynchronously, the pandas' food supply does not disappear. But human pressure has in particular resulted in the loss of the lower-altitude species of bamboo in many areas, in effect removing the pandas' emergency alternative rations. The animals can and do switch between different bamboo food types, as this study showed; but it also showed that they have very little spare time to spend in searching for and gathering their food. They are near the limits of their existence, for several reasons. The remedies for the situation will form the basis of the research team's next report. We shall await it just as eagerly. □

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Matter of a letter

Stillman Drake

The Galileo Affair: A Meeting of Faith and Science. Edited by G. V. Coyne, M. Heller and J. Zyciński. *Specola Vaticana, V-00120 Stato Città Del Vaticano, Vatican City State; 1985. Pp.179. Pbk \$7 (individuals), \$9 (libraries).*

In 1979 the Pope expressed concern that Galileo had suffered at the hands of persons and instrumentalities of the Catholic Church. Citing a passage from Galileo's open letter of 1615 on religion and science, addressed to the Grand Duchess of Tuscany, he implied that Galileo's theological position was sounder than that of his judges at his trial for suspected heresy, in 1633. The Pope proposed a thorough re-examination of the Galileo affair, placing blame wherever it historically belonged.

Several publications at Vatican City have subsequently appeared. Among the first was the complete official text of all documents relating to the affair now extant at Rome. Most recent is this book, the proceedings of a conference held at Cracow in 1984 and attended by historians, philosophers, scientists and theologians. History of the Galileo affair occupies the first section, followed by sections on Galileo and science, and on cultural repercussions of the Galileo affair.

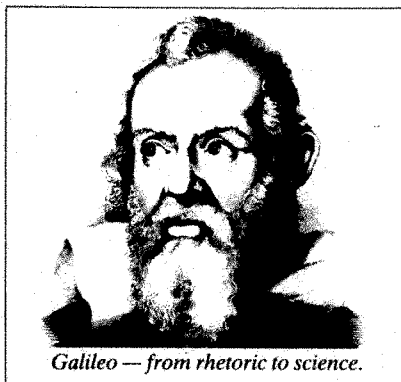
As translator of Galileo's letter of 1615 and of his books, I oppose the claim (p.30) that the former "is filled with assertions that the Earth's motion, both diurnal and annual, can be conclusively proved by

necessary demonstrations based on sense experiences". No such categorical assertion was made. The part of the letter cited says no more than that after decades of unopposed study, Copernican astronomy was being attacked by philosophers just when it was beginning to appear well-founded by manifest experiences (telescopic) and necessary demonstrations (mathematical).

One such set of foundations was the phases of Venus announced at the beginning of 1611. Another was eclipses of Jupiter's satellites, published in 1613 with some predictions by Galileo and his remark that motion of the Earth had to be considered in calculating them. His calculations showed that the Earth was not at, or even near, the centre of Jupiter's orbit. The scholar concerned is also mistaken in assuming that Galileo alluded to his explanation of tides, which involved no measurements, mathematical calculations or predictions subsequently verified observationally.

In 1615 Galileo openly stated his Copernican belief; no action had yet been taken by theologians on the matter. The purpose of his letter was to show that any action would be untimely, not to prove any scientific conclusion. In 1616 an edict regulating Copernican books was issued. Thereafter Galileo discussed the matter only hypothetically, having promised in his letter to abide by Church action if, contrary to his advice, any were taken.

Thorough re-examination of the Galileo affair as proposed by the Pope is



Galileo — from rhetoric to science.

Mary Evans

still far from accomplished. Another scholar at Cracow writes (p.137): "The revolutionary ideas of Galilean astronomy were due to fortunate conjectures and insights which had no critical empirical confirmation before 1838". No scientist altered his astronomy because of Bessel's observations. Moreover Galileo's calculations of satellite eclipses were not fortunate conjectures. Whether philosophers should reject them as critical empirical confirmation of the Earth's place and motions is at least questionable. Philosophy and rhetoric are no longer science, as they were until Galileo separated them from it.

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AIDS: the scientific story so far

June Goodfield

A Strange Virus of Unknown Origin. By Jacques Leibowitch. Translated by Richard C. Howard. *Available Press (Ballantine, New York); 1985. Pp.172. Pbk \$4.95 (United States), \$6.50 (Canada).*

ACQUIRED immune deficiency syndrome, AIDS, struck medical consciousness at a time when, as Robert C. Gallo points out in his foreword to this book, prominent scientists were predicting the end of the great human infectious epidemics. Though the disease had certainly already been present in a few special localities, it appeared where it might have been least expected, namely in technologically advanced, relatively healthy societies.

The medical community might have been taken by surprise, but the cause of AIDS was discovered with a speed probably unparalleled in the history of infectious diseases. A new virus, unique to human beings, preferentially attacks the helper T-lymphocytes, the very cells whose activity is crucial in the mounting of an adequate immune response. The immune deficiency thus provoked lays the victim open to a whole variety of invariably fatal opportunistic infections. As they watch the spread of AIDS gather momentum, medical scientists are realizing that, far from being over, a new, dangerous period of infectious epidemics may just be starting.

We are fortunate that a scientist involved in research into this disease from its very beginning has now written this excellent, slim book. Jacques Leibowitch is a clinical immunologist who has been both close to the problem and totally committed to its solution. Here he successfully demystifies AIDS, recognizing, as we all should, that to know your enemy well is the first step towards victory. Taking us on a survey through the history and origins of the epidemic, he presents a comprehensive and authoritative clinical and epidemiological picture that culminates with sections on prevention and the prospects for treatment — not at all bright as of now.

Everyone from professional scientists to the general public will find this book of great value. It is marred, however, by one stylistic fault. Though at the beginning the writing is fluent and witty, the book finishes in a series of choppy, condensed paragraphs covered liberally by sub-headings, so that it takes on the quality of a briefing paper for a select committee. □

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Rural poverty breeds fertility

from Alok Vajpayee

The conditions of village life in rural India create the need for large families. Reduced fertility will come only with improvement.

THE Family Planning Programme (now known as the Family Welfare Programme) has been in operation in India since 1953. The main approach is to fix targets for sterilization operations and other methods of contraception for each area and to press the responsible authorities to achieve them.

This approach is unnatural, because most villagers in India need large families. Motivating them to adopt family planning is a difficult task. The more primitive the way of life the larger the family they need. This is a matter of survival, not simply an option as many villagers well realize.

Of course, the villages are changing, but very slowly.

The urbanization of the villages is followed by a change in life style. And when a village becomes a town the residents will need smaller families. The rural population of India resides in some 576,000 villages, 55 per cent of which contain fewer than 500 persons and 23 per cent of which have between 500 and 1,000 persons (these data and others in this article are from *Health Statistics of India — 1984*, published by the Ministry of Health and Family Welfare). The smaller the village, the greater will be the need for a large family.

Rural life is harder than urban life, which is why a rural family needs more working hands. In the villages, a family must itself do almost all the work required for its survival. In other words, a rural family is more self-sufficient than an urban family. Take, for example, water supply and the disposal of domestic liquid and solid wastes. For both tasks, an urban family does not have to exert itself at all. Water is supplied to houses through pipes and, for the disposal of liquid wastes and excreta, there is a public sewer system.

Services

In villages, these tasks demand careful attention. According to official statistics, in 1984 some 83,000 villages were without even a single source of year-round drinking water. People have to go and carry water, sometimes from a source miles away from the home. For defaecation, almost 100 per cent of the rural population goes to the fields, outside the villages. In the absence of a proper drainage system and a piped-water supply, because of the space available in village houses, the financial constraints and the lack of technical expertise in the villages for the con-

struction and maintenance of sanitary latrines, it is better for them to go into the open for defaecation than to construct and maintain a latrine in their houses.

In the cities one can purchase all types of services; there are laundry services for washing clothes, crèches to look after the children . . . and many other types of service. In the villages, by contrast, such services cannot be purchased; instead, those considered essential must be performed by the family itself.

Electricity makes life extremely comfortable and saves much human and animal labour. But by 1983, about 275,000 villages had not been electrified while even in those with electricity, the supply is erratic and is frequently interrupted.

Agriculture is the main occupation of the villagers and needs a great deal of human labour, at least as practised by most Indian farmers. For the purchase of household items, clothes, utensils and so on — the majority of villagers depend on weekly markets which are held in larger villages, or they may go to a nearby city. But there is a great problem of transport. Of the villages of India, 53 per cent were not connected by all-weather roads in 1982.

So therefore, all of the tasks performed by a rural family require a great deal of human labour and time, and only a large family is suitable for such a life style.

In the villages, life and property are much more insecure than in the cities. Robberies, land disputes, murders, thefts and familial rivalries are common, but the nearest police station is usually too far away to be contacted quickly, while the law enforcement agencies are lax. At any one time, most of the police force is engaged on law enforcement in the cities, handling strikes and regulating traffic, for example. Even the mass media have an urban bias. A quarrel or theft in a metropolitan city may hit the headlines of a national daily while a major atrocity on villagers may go unnoticed. Most of the national newspapers and magazines suffer from myopia.

A large family gives a sense of security against such happenings as well as 'insurance' against natural calamities such as floods, fires and the like.

The very old and the very young, the sick and pregnant need special care and attention. A large family can deal with sudden crises such as illness and the normal stresses of birth and old age without

help from outside. A young grandchild may be assigned the duty of watching grandma and helping her, or vice versa.

In the villages, professional expert help cannot be easily obtained. According to an estimate made in 1979–80, only 10–15 per cent of births in India were conducted by trained attendants. Most of the rural population depends on quacks for treatment.

Mortality

Inevitably, the death rate is higher in the villages than the cities. In 1982, the crude death rate for the rural population was 12.9 per 1,000 population, while in the urban areas it was 7.4 per 1,000 population. There is a positive correlation between death rate and birth rate; the higher the mortality, the greater will be the fertility. The collapse or removal of a family member in a small family of four or five, with only a few relatives, will have far more serious consequences than in a large 'unplanned' family.

Mortality in childhood, especially infant mortality and under-five mortality, is very high in the villages. The infant mortality rate in 1980 was 124 per 1,000 live births in the villages and 65 per 1,000 live births in urban areas. The under-five mortality rate in 1980 was 46.1 per 1,000 for rural areas and 22.2 for the urban areas. The smaller the chances of survival of a child, the higher will be fertility. Villagers feel that they should produce too many children if they are to be sure that at least one or two sons will survive to provide care for their parents in old age.

So if the government wishes family planning to be a success rather than a mere slogan, it must provide intensive maternal and child health facilities, giving sufficient health coverage to the mother and her new-born. Child-health is the best motivator of family planning.

As things are, the villagers of India need large families. Overall socio-economic uplift and rural development will bring about a change in life-style that will diminish the need and will ultimately reduce fertility. Rural electrification, better transport facilities for rural areas, the construction of good roads, better medical facilities, the provision of a piped water supply and the like are thus the long-term agents of demographic change. □

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Control of eukaryotic messenger RNA synthesis by sequence-specific DNA-binding proteins

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The enzymatic machinery that carries out RNA synthesis provides the cell with the means to adjust the patterns of transcription in response to environmental and developmental signals. In eukaryotes, this regulation is mediated in part by promoter-specific transcription factors, which are DNA-binding proteins with the ability to discriminate between distinctive DNA sequence elements found in the promoter regions of different genes. The presence of these factors bound to DNA enables other components of the transcriptional machinery, including the RNA polymerase, to initiate transcription with selectivity and accuracy.

THE principal method of identifying the DNA sequence elements that control transcription in eukaryotes has been to mutagenize DNA sequences near the start of transcription, then to test the altered templates either by reintroduction into the cell or by introduction into some experimental substitute, such as an *in vitro* transcription system. Accumulation of new RNA or protein is measured after a period of time, and from this an inference is made about the effect of sequence changes on promoter strength. These studies have shown that, although promoters for protein-coding genes vary in sequence, they are organized according to a common plan (Fig. 1).

Many promoters have an element called a TATA box, with a consensus sequence 'TATAAA', located 25–30 base pairs (bp) upstream from the beginning of the transcribed sequence (for review, see ref. 1). Mutation in this region generates 5' (upstream) heterogeneity in the transcripts^{2–4}, although the overall level of RNA synthesis may not be significantly reduced. Further upstream from the start site of transcription is a region containing one or more additional promoter elements. Some upstream elements, such as the 'CCAAT' or 'GGGCGG' homologies, have now been found in many different promoters, whereas others, such as the metallothionein metal regulatory element^{5–7}, or the heat-shock regulatory element⁸, have a more specialized role. As we shall establish, upstream promoter elements are a principal target for the action of promoter-specific transcription factors.

The activity of many promoters is modulated by an enhancer, which is a separate regulatory element. The enhancer must be on the same molecule of DNA, but can be 1,000 bp or more from the promoter, and may be located either upstream or downstream from the transcription start site (Fig. 1)^{9–12}. The prototype enhancer is found in simian virus 40 (SV40), a DNA tumour virus^{9,10,12–14}, but control elements with similar properties have since been found in other viruses and in the cellular genome (for example, refs 11, 15–17). Some enhancers are tissue-specific^{18–26}, whereas others mediate transcriptional responses of certain genes to steroid hormones²⁷.

Protein factors in transcription

The understanding of promoter function gained by analysis of DNA sequences has certain limitations, which can be overcome only through identification, purification and characterization of the proteins required for transcription. One would like to know how the proteins that bind to promoter and enhancer elements bring about transcriptional regulation and, in particular, to understand how the abundance and activity of transcription factors change through cell growth and development.

The enzyme responsible for mRNA synthesis, RNA polymerase II, differs from prokaryotic RNA polymerase holoenzyme in that it seems to lack any inherent ability to recognize promoters in an *in vitro* reaction. It has recently become clear that crude extracts from eukaryotic cells contain factors that impart promoter specificity to the purified RNA polymerase II and allow *in vitro* initiation of transcription at the same start sites as are used *in vivo*^{28–33}. Some of these factors seem to be required for initiation at all promoters^{34–40}, whereas others are required for initiation at certain promoters but not others. Many of these latter, promoter-specific, factors bind DNA sequences in their respective target promoters. DNase footprinting⁴¹ has been used to measure this site-specific binding in several instances^{39,40,42}. In the ideal case, the binding and transcriptional properties of a factor can be related experimentally, with stimulation of transcription requiring sequences within the binding site, and moreover, with binding and transcription stimulatory activities co-purifying during fractionation of the extract.

The promoter-specific factor Sp1

Sp1, a protein possessing many of the characteristics described above, is a factor obtained from cultured human cells and required for transcription of SV40 (ref. 37). Activation of transcription by Sp1 requires a well-defined upstream element, which in SV40 occupies a region 50–100 bp upstream from the start site for early viral RNA transcription^{14,43–45}. When this sequence is deleted, the early promoter is no longer able to respond to Sp1 (ref. 42) and, inversely, when the sequence is fused to a heterologous TATA box element, the hybrid promoter becomes Sp1-responsive (D. Gidoni and R.T., unpublished). The Sp1 target element seems to function bidirectionally⁴⁶, and plays a role in directing late viral transcription from the opposite strand of the DNA^{14,42,45,47–51}.

Binding experiments show that purified Sp1 interacts directly with the sequences required for the transcriptional response⁴². The sequence asymmetry of the Sp1-binding site is a distinctive feature; unlike the binding sites for prokaryotic transcription

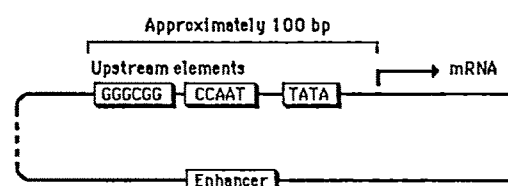


Fig. 1 Arrangement of DNA sequence elements controlling transcription of a typical eukaryotic protein-coding gene. The enhancer may be located upstream or downstream of the site of transcriptional initiation.

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factors, such as the lactose operon repressor, the phage λ repressor, or cyclic AMP receptor protein (for review, see ref. 52), there is no suggestion of dyad symmetry in the region. Sp1-DNA contacts detected by dimethylsulphate protection fall within a sixfold repeated sequence moiety ('GGGCGG'; ref. 53). Sp1 forms strong contacts with three of these GC boxes and weakly contacts two others (Fig. 2). Contacts have so far been detected only in the major groove of the DNA and on one strand. Alteration of individual GC boxes by oligonucleotide-directed mutagenesis suggests the presence of five independently bound protomers within the overall region, with each Sp1 protomer contacting one GC box and protecting ~20 bp from DNase (D. Gidoni, J. Kadonaga, H. Barrera-Saldaña, K. Takahashi, P. Chambon and R.T., unpublished).

Other Sp1 binding sites

When Sp1 was first isolated, its interaction with SV40 was thought to be unique. Recently, however, Sp1 has been shown to be required for *in vitro* transcription of several genes of herpes simplex I virus, including immediate early gene 3 (ref. 95) and a delayed early gene, thymidine kinase⁹⁶. The pattern of Sp1 binding differs from that in SV40, with each control region in the herpes virus containing multiple separate 'unit sites' for Sp1 binding (Fig. 3). Each unit site covers ~20 bp and presumably accommodates a single Sp1 protomer. Binding at the thymidine kinase promoter is particularly interesting because Sp1 seems to interact with the 'distal elements' previously defined by mutational analysis^{54,96}. Activation seems to require both Sp1 and a second, as yet unidentified, factor that binds in the region between two Sp1-binding sites.

In addition to these viral genes, several cellular sequences also interact with Sp1. A DNA segment from the monkey genome, which functions *in vivo* as a bidirectional promoter element⁵⁵, contains two Sp1-binding regions, one of which, the β -site, is shown in Fig. 3⁵⁶. Multiple Sp1-binding sites are also found in 5' flanking sequences of the mouse dihydrofolate reductase (*dhfr*) gene (W.S.D., S. Sazer, R.T. and R. Schimke, unpublished; for sequence, see ref. 57). The monkey β -binding region is relatively large and may be a complex binding site, like that of SV40. The mouse *dhfr* promoter, by contrast, shows unit binding sites reminiscent of those found in the herpesvirus promoters. All the Sp1-binding regions contain one or more perfect copies of the GC box hexanucleotide GGGCGG, which may be present in either orientation with respect to transcription. Not all GC boxes bind Sp1 equally well, and sequences outside the core hexanucleotide seem to modulate the efficiency of binding. A consensus sequence, compiled from 19 individual strong binding sites, is shown in Fig. 3⁹⁷.

Recently a number of additional GC box-containing promoters have been described. These include promoters for cellular genes, such as human metallothionein-1A (ref. 58), hamster hydroxymethyl-glutaryl coenzyme A reductase⁵⁹, mouse hypoxanthine phosphoribosyl transferase⁶⁰, mouse adenine phosphoribosyl transferase⁶¹, human adenosine deaminase⁶², and rat type II procollagen⁶³, as well as promoters for other viral genes, including herpes simplex I immediate early genes 4 and 5 (ref. 95) and AIDS retrovirus (refs 64-66; K. Jones, J. Kadonaga, P. Luciw and R.T., unpublished). Some of these putative binding sites have been tested in our laboratory for their ability to bind Sp1, but in most cases binding is merely predicted from the DNA sequence.

No shared regulatory feature is evident among the diverse promoters in which actual or potential Sp1-binding sites have been found, and thus we cannot at present explain why some promoters have Sp1-binding sites and others do not. It is possible that Sp1 provides a framework to which other regulatory proteins may bind; it is also possible that Sp1 provides a basal, constitutive level of transcription that is then modulated by other positive or negative regulatory factors. The occurrence of binding sites in the transcription control regions of unrelated viruses suggests that Sp1 is not only widespread among different cells but also

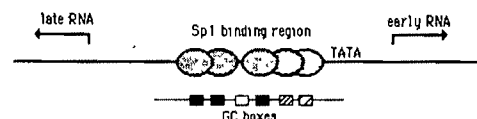


Fig. 2 The Sp1-binding region of SV40. Ellipses represent Sp1 molecules; darker shading indicates higher-affinity binding. Filled GC box moieties are fully protected from dimethylsulphate modification, the hatched GC boxes are partially protected and open GC boxes are unprotected. The initiation sites for late RNA are heterogeneous; only the most Sp1-proximal is drawn.

readily available within the cell to facilitate rapid viral gene expression on infection.

Heat-shock transcription factor

A factor that is similar enzymatically to Sp1 is involved in directing the heat-shock transcriptional response. When cells of any organism are exposed to abnormally high temperature, several heat-shock mRNAs are induced. In *Drosophila*, and apparently in mammalian cells as well, the induction of transcription is mediated by a conserved heat-shock regulatory sequence found in the upstream region of the promoters for heat-shock RNAs (ref. 8; for review, see ref. 67). A heat-shock transcription factor (HSTF) has been isolated from *Drosophila* that seems to interact with this element in a manner analogous to the interaction between Sp1 and its recognition sequence⁴⁰. In the promoter for the relative molecular mass (M_r) 70,000 heat-shock protein, HSTF binds 40-95 bp upstream from the TATA box (Fig. 4). Like Sp1, the HSTF binds to its target sequence in the absence of RNA polymerase II and is promoter-specific, stimulating transcription of the heat-shock gene but not, for example, the actin 5C gene⁴⁰.

One of the attractive features of the heat-shock system is that the *in vivo* role of the factor-sequence interaction has also been analysed. Digestion of chromatin with exonuclease indicates that sequences in the HSTF binding site are accessible in normal cells, but protected, presumably by HSTF, in heat-shocked material^{68,69}. HSTF can be isolated in more-or-less equivalent amounts from normal and heat-shocked cells; therefore, its appearance at the promoter during the heat-shock response probably reflects a change in localization or properties rather than *de novo* synthesis. The nature and mechanism of this change remains to be elucidated.

Other DNA binding factors

The degree to which the effects of upstream elements are reproduced *in vitro* varies depending on the promoter and the way in which the extract is made⁷⁰. With some promoters, such as rabbit β -globin, upstream sequences required for *in vivo* expression have had no effect *in vitro*⁷¹. In other instances, upstream sequences have had a substantial effect on *in vitro* transcription. In addition to the promoters mentioned specifically above, upstream sequences have been shown to be required for maximal *in vitro* transcription of the adenovirus-2 major late promoter^{70,72}, the silkworm fibroin promoter⁷³, one of the sea urchin histone promoters⁷⁴ and the *Drosophila* alcohol dehydrogenase promoter⁷⁵. The dependence on upstream sequences implies the presence of corresponding factors in cell extracts, and at least in the case of the alcohol dehydrogenase and adenovirus promoters, it has been possible to detect the binding of these factors directly, by DNase footprinting (ref. 75, W. Morgan and R.T., unpublished; R. Roeder, personal communication). Although not yet correlated with an upstream element, the elevated histone promoter transcription seen with extracts from S-phase cells may reflect the functioning of yet another promoter-specific factor⁷⁶.

In addition to proteins that bind upstream sequences, a factor that binds the region containing the TATA element has been

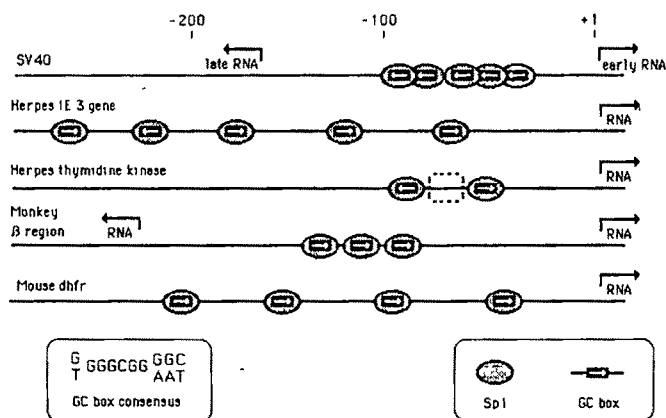


Fig. 3 Different arrangements of Sp1-binding sites. Ellipses represent Sp1 molecules, with darker shading indicating higher affinity binding. Arrows within Sp1 symbols represent GC boxes, with the arrow pointing in the 5' → 3' direction on the G-rich strand of DNA. For clarity, only those GC boxes that actually bind Sp1 are drawn. Some promoter regions have additional GC boxes that do not bind Sp1, possibly because of steric hindrance from Sp1 at adjacent sites. The GC box consensus sequence was obtained by comparison of 19 strong binding sites⁹⁷. Dotted rectangle in herpes thymidine kinase gene represents an additional factor binding between Sp1 molecules.

identified both in *Drosophila*³⁹ and in mammalian cell extracts (ref. 77; W. Morgan and R.T., unpublished; M. Sawodogo and R. Roeder, personal communication). The *Drosophila* factor interacts with several *Drosophila* promoters, including those of the actin, histone H3 and histone H4 genes³⁹. Binding (as assayed by DNase footprinting) occurs in the absence of upstream factors, with the protected region extending from about -40 to +25.

Initiation of transcription

In addition to the site-specific DNA-binding proteins described here the actual initiation of transcription requires RNA polymerase II and at least three other enzymatic factors (refs 34–39; D. Reinberg and R. Roeder, personal communication). Under appropriate conditions, these proteins form a complex at the promoter before initiation of transcription.

Various kinetic and order-of-addition experiments have been of some help in elucidating the steps involved in complex assembly. Davison and coworkers⁷⁷ showed by an indirect depletion assay in a mammalian system that DNA fragments containing a functional TATA box homology could sequester an essential transcription factor from the reaction. Fire and coworkers⁷⁸ carried this approach further and developed a model for ordered assembly of a complex at the adenovirus major late promoter. Their results suggest that factors present in two fractions bind first, in an indistinguishable order, followed by association of RNA polymerase II and, in a final rapid step, factors present in a third fraction. In the absence of nucleoside triphosphates, no initiation occurs and the complexes are stable for at least an hour. Although it is important to interpret these experiments cautiously, because separation and purification of the components might cause events to happen differently *in vitro* than in the cell, this sort of experiment seems to be the best available tool for elucidating the roles of the multiple factors that participate in the initiation reaction.

A marked inhibition of RNA polymerase II activity is seen when β - γ -imido ATP (AMP-PNP) is substituted for ATP in the promoter-directed *in vitro* transcription reaction⁷⁹. The ATP analogue, which cannot be hydrolysed at the β - γ position, apparently fails to satisfy a requirement for hydrolysable adenosine nucleotide during the initiation reaction. AMP-PNP can be hydrolysed normally at the α - β position, and in the post-initiation elongation phase of the reaction can be incorporated normally into RNA. Addition of deoxy ATP to a reaction containing AMP-PNP restores the ability to initiate even though

the deoxy compound cannot be incorporated into the nascent RNA chain^{37,80}, clearly demonstrating that the requirement for β - γ hydrolysis is separate from elongation. The requirement for a hydrolysable adenosine compound is independent of which base is present at the 5' terminal position in the RNA, and has been seen only with RNA polymerase II and not with the other eukaryotic RNA polymerases. As yet, it is not known whether hydrolysis is carried out by the polymerase itself, or by one of the additional factors involved in initiation.

How factors influence transcription

The role of upstream factors should be viewed in the context of their interaction with other components of the transcriptional machinery. In the simplest case, an upstream factor might bind next to the transcriptional initiation site, thus inducing a conformational change in either the DNA or in other proteins that make up the initiation complex (Fig. 5a). An example of a promoter that may be activated by this mechanism is that for the *Drosophila* *M*, 70,000 heat-shock protein, where the most proximal heat-shock recognition sequence is only 15 bp from the TATA box. In other heat-shock promoters, however, as well as many of the Sp1 responsive promoters (refs 67, 81; see also Fig. 3) the upstream factor binding site may be situated farther from the initiation site, making direct protein-protein contact less likely as a mechanism for transcriptional activation. Moreover, many promoters have multiple binding sites for upstream factors, with the most distal sites clearly out of range for direct contact with proteins bound at the initiation site.

There are several possible models that could explain the apparent ability of upstream factors to act at a distance. After initial binding to the recognition site, a factor might shift positions to make productive contact with other proteins at nearby sites (see Fig. 5b). A necessary consequence of this model is that an input of energy would probably be required to allow the factor to dissociate from its high-affinity binding site and move along the helix. An alternative model is that the upstream factor might remain in place but induce a conformational change in the DNA helix, such as an unwinding; in this case, the arrow in Fig. 5b can be taken to represent the propagation of the conformational strain through the DNA. A different possibility is drawn in Fig. 5c, where a second factor binds alongside the first to create a continuous scaffold of protein. This interstitial binding may occur in herpes thymidine kinase, where a second factor has recently been found to bind between the two Sp1 sites (see Fig. 3). Another model supposes that factors bound at multiple upstream sites allow DNA to condense into a compact nucleoprotein structure (Fig. 5d). There is as yet no direct evidence for such a structure, although similar models have been proposed to explain the role of multiple binding sites involved in site-specific recombination and DNA replication in bacteria⁸² and for enhancer activation of promoters in eukaryotes⁸³.

Some upstream elements function bidirectionally. This might be expected with the heat-shock element, where the consensus recognition sequence has a dyad symmetry. Similarly, in herpes thymidine kinase, two Sp1 binding sites are present in opposite orientations, making it easy to explain the activity of the upstream elements when their overall orientation is inverted⁸⁴. The properties of the SV40/Sp1-binding region are more difficult

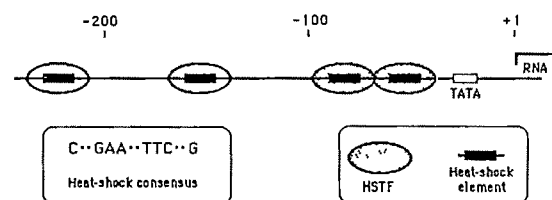


Fig. 4 Arrangement of HSTF binding sites in the promoter for the *Drosophila* *M*, 70,000 heat-shock protein. Stippled ellipses cover region where binding has been reported; open ellipses indicate regions of potential HSTF binding further upstream from the initiation site.

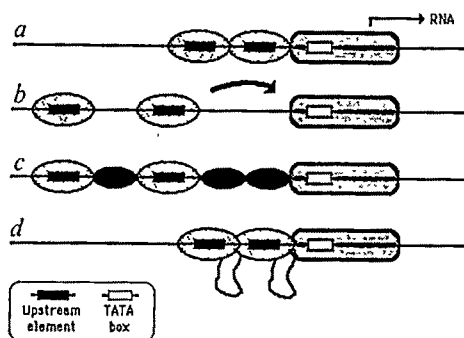


Fig. 5 Models for the action of promoter-specific factors. *a*, Transcription factors (stippled) bound to upstream elements, in direct contact with other proteins (shaded) bound at the initiation site; *b*–*d*, action at a distance. *b*, Upstream factor shifts position to contact proteins at initiation site, or alternatively, alters conformation of DNA, with effect felt at initiation site. *c*, Additional proteins bind interstices to create a continuous scaffold of protein in the promoter region. *d*, Transcription factors condense into a compact nucleoprotein structure, with looping-out of intervening DNA.

to explain, however, because despite its marked sequence asymmetry, the region is clearly involved in the genesis of transcripts from both viral strands^{14,43–45,47–51} and retains its ability to direct early RNA synthesis when inverted⁴⁶. This apparent paradox has not been resolved, although a simple possibility that cannot yet be excluded is that activation of transcription in opposite directions occurs by different and independent mechanisms.

Activation of transcription by enhancers

One persistent question in the field is whether enhancers are fundamentally different from upstream promoter elements of the sort that interact with Sp1 or HSTF. Clearly, as more experiments have been done, many upstream promoter elements have been found to have characteristics once thought to be distinctive properties of enhancers, such as bidirectionality and a degree of flexibility in spacing^{42,46,81,84}. So far, however, only enhancers have shown an ability to influence transcription over distances of a kilobase or more, and to work upstream or downstream of the promoter.

It seems probable that enhancers, like promoters, will turn out to be regions of interaction for site-specific DNA binding proteins. This has been shown directly for the enhancer-like glucocorticoid response elements of murine mammary tumour virus and the human metallothionein II_A gene, both of which bind glucocorticoid receptor^{5,85–87}. It is uncertain whether enhancers are sites for binding of a single protein or, like promoters themselves, are sites for assembly of a multiprotein complex, with some proteins binding directly to the DNA and others involved in other aspects of the reaction. There is evidence that different enhancers compete with each other for limiting components in the cell^{87,88}, which is consistent with the idea that various cellular and viral enhancers interact with at least some common factors.

Several groups have been successful in showing an effect of the SV40 enhancer in an *in vitro* system where transcription is activated 10-fold or more under optimal conditions^{89–92}. It is not yet certain, however, that the activation is caused by the enhancer *per se*, and not to other sequences, such as elements of the late promoter, that are present in the same region of SV40. In addition, the unique and distinguishing feature of enhancer activity, the stimulation of promoters over distances of ≥ 1 kilobase (kb), and from downstream of the gene, has not yet been demonstrated with an *in vitro* system.

Little more is known now about how enhancers transmit their effects over distances than when these elements were first discovered. Assuming that enhancers act at the level of initiation of transcription^{93,94}, it seems evident that they must facilitate some rate-limiting step; moreover, if they are binding sites for proteins they should follow the ordinary rules governing pro-

tein-DNA interactions. The models discussed previously for upstream promoter elements are therefore likely to be applicable to enhancers as well. A looping out of intervening DNA, in particular, could explain several features of enhancer-promoter interactions⁸³, and would allow a DNA-binding protein to exert its effect over any distance.

Conclusions

In this review we have chosen to focus on experiments involving biochemical fractionation and analysis of proteins from cell extracts. To keep this review brief, we have largely omitted discussion of several valuable alternative approaches. These include the use of enzymatic and chemical probes for analysis of chromatin structure in promoter and enhancer regions, as well as attempts to obtain mutants in the genes encoding trans-acting factors. Similarly, we have not discussed trans-acting factors such as the adenovirus E1A protein, which seem to modulate transcription by a mechanism other than sequence-specific DNA binding.

Although many details remain to be established, recent work clearly indicates the existence of promoter-specific transcription factors that recognize distinctive sequence elements found 40–100 bp upstream from the initiation site in eukaryotic protein-coding genes. Several such factors have been isolated and each factor recognizes a different sequence element. Binding activates transcription and allows the transcription machinery to discriminate between different promoters in an *in vitro* reaction. Within a promoter there are often multiple binding sites for the same or different factors, and the distance to the start site of transcription can vary significantly, suggesting that a mechanism exists for transmitting the activating signal through at least short stretches of intervening DNA. In addition to the sequence-specific DNA binding proteins, several other factors required for transcriptional initiation have been partially characterized, and these proteins, together with RNA polymerase, assemble via an ordered pathway into a functional preinitiation complex.

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ARTICLES

Residence time of thorium, uranium and lead in the mantle with implications for mantle convection

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The residence times of thorium, uranium and lead in the upper mantle are estimated at ≤ 600 Myr, and should be similar for related elements that are strongly concentrated in the continental lithosphere. Their upper mantle abundances are close to steady state and are mostly supported by entrainment of material from a lower mantle layer with long-term convective isolation. These lithophile trace elements and rare gases, such as ^3He , are effective chemical probes of the deeper mantle.

ONE of the main aims of geochemistry is to quantify the mass transfer processes that have occurred between different portions of the Earth, such as those responsible for the generation of the continental crust and atmosphere. The development of the continents, for example, has resulted in a marked transfer of the principal heat-producing elements (K, U and Th) and related large-ionic-radius trace elements from the mantle to the crust. A limit on the proportion of mantle involved in forming the continents has been imposed from the mass balance of those parent and daughter atoms involving the radiogenic isotopes of Nd, Sr, Hf and Pb (refs 1-4). These geochemical considerations together with those of the primordial and radiogenic components of the rare gases⁵ have been central to arguments as to whether convection extends throughout the whole mantle or is separated into two layers. In the latter case of layered convection, attention has been drawn to the requirement for transport of at least heat and He across the separating boundary layer⁶.

The depleted character of the upper mantle is evident in both the elemental and isotope compositions of mid-ocean ridge basalts (MORB). In particular, trace elements that strongly partition into silicate melts (bulk solid/liquid distribution coefficient $D_1 \ll 1.00$), such as Cs, Rb, K, Th, U and light rare earths, are estimated to be present at $\leq 20\%$ of their initial

abundances¹⁻⁴. This depleted character is evident not only in Recent MORB and Phanerozoic ophiolites but also in some Archean komatiites and basalts^{7,8}, indicating that part of the mantle has been depleted for $\sim 3,000$ Myr. Because elements with the lowest D_1 values are extracted more efficiently than those with larger D_1 during melting, it can be anticipated that some relationship exists between transport, distribution coefficient (D_1), residence time (t_r) and degree of mantle depletion.

In this article, we suggest that limits can be placed on the residence times of Pb, Th and U in the mantle, and that the upper mantle has close to steady-state concentrations for these elements. The results have important implications for the origin of chemical and isotope heterogeneity in the mantle, the degree of boundary-layer interaction in a convectively-stratified mantle, and the early differentiation of the terrestrial planets.

Residence times

Th and U are highly incompatible elements that are strongly depleted in the Earth's upper mantle. The Th/U ratio or κ value ($^{232}\text{Th}/^{238}\text{U}$ ratio) of the bulk Earth has been estimated at ~ 3.85 from the Pb isotope compositions of conformable lead ore deposits^{9,10}, in good agreement with the value of 3.9 ± 0.1

Table 1 Th/U ratios in oceanic basalts from Pb and Th isotopes

	$\bar{\kappa}_{\text{Pb}} \pm 1\sigma$	n	Ref.	$\kappa_{\text{Th}} \pm 1\sigma$	n	Ref.
MORB						
Mid-Atlantic Ridge	3.78 ± 0.07	71	17, 24, 25, 36, 41, 42	2.5	—	43
East Pacific Rise	3.73 ± 0.06	31	17, 24, 25, 41, 42	2.5 ± 0.2	18	14
Mid-Indian Ridge	3.89 ± 0.11	21	17, 24, 25, 44			
Juan de Fuca-Gorda	3.73 ± 0.04	24	45			
Seamounts						
North-east Pacific	3.73 ± 0.03	18	45			
Islands						
Hawaii	3.84 ± 0.04	55	11, 17, 46, 47	3.0 ± 0.2	18	48
Iceland	3.81 ± 0.03	39	25, 49, 50	3.0 ± 0.2	17	51
St Helena	3.75 ± 0.02	14	17, 52			
Australas	3.75	5	53			
Ascension	3.81 ± 0.02	16	17, 52, 54			
Azores	3.84	5	17	2.6	4	55
Bouvet	3.84	3	17			
Canaries	3.92 ± 0.03	40	17			
Marquesas	3.98 ± 0.04	10	53			
Guadalupe	3.99	4	17			
Tristan de Cunha	4.17	8	17, 52	3.7	5	55
Gough	4.20	8	17			
Kerguelen	4.24 ± 0.12	24	56			
Walvis Ridge	4.25	9	57			

n = number of samples. $\bar{\kappa}_{\text{Pb}} = \{^{208}\text{Pb}^*/^{206}\text{Pb}^*\}(e^{\lambda^{238}\text{T}} - 1)/(e^{\lambda^{232}\text{T}} - 1)$ where T is the age of the Earth (4,550 Myr), λ_i the decay constant of parent isotope i , and $^{208}\text{Pb}^*/^{206}\text{Pb}^*$ is corrected for primordial Pb (ref. 16). $\kappa_{\text{Th}} = (\lambda_{238}/\lambda_{232})(^{232}\text{Th}/^{230}\text{Th})$ where $(^{232}\text{Th}/^{230}\text{Th})$ is an activity ratio.

deduced from igneous rocks of various ages^{11,12}. The latter value of 3.9 is adopted here for the bulk Earth Th/U ratio, but a precise value is not critical to the central argument.

The present-day Th/U ratio of the upper mantle may be estimated from Th and U abundances or from $(^{232}\text{Th}/^{230}\text{Th})$ activity ratios of MORB, derived by melting the mantle beneath spreading ridge axes. Measured $(^{232}\text{Th}/^{230}\text{Th})$ activity ratios from the East Pacific Rise and other MORB (Table 1) correspond to $\text{Th}/\text{U} \approx 2.5$ in their mantle sources, however; the observation that $1 < (^{230}\text{Th}/^{238}\text{U}) < 1.3$ for most MORB also suggest that Th enters the melt phase preferentially over U and that $D_{\text{Th}} < D_{\text{U}}$ (refs 13, 14). This is consistent with Th/U concentration ratios reported by Jochum *et al.*¹⁵ which range from Th/U = 1.5 to 3.1 as the Th content increases, and should, therefore, be an upper limit on the Th/U ratios of their sources. When taken together, this implies that $\text{Th}/\text{U} \leq 2.5$ in the upper mantle sampled at spreading ridges, or around half of any estimate for the bulk Earth.

An additional estimate of Th/U, albeit a time-integrated average over Earth history, can be calculated from the Pb isotope compositions of oceanic basalts; values of $\bar{\kappa}_{\text{Pb}}$ are derived from the radiogenic additions to ^{208}Pb and ^{206}Pb from their respective parent isotopes ^{232}Th and ^{238}U making allowance for primordial Pb (ref. 16) (Table 1). Most MORB show a restricted range from 3.65 to 3.89, with an average value of ~ 3.75 (Table 1); ocean-island basalts exhibit a larger range ($3.7 < \bar{\kappa}_{\text{Pb}} < 4.2$). Thus, the Th/U ratio that the Pb in oceanic basalts has 'seen' over its history ($\bar{\kappa}_{\text{Pb}}$) is higher than the deduced $\text{Th}/\text{U} < 2.5$ of the upper mantle, but close to the bulk Earth value of ~ 3.9 .

At first sight, these relationships^{11,17,18} appear paradoxical because the low Th/U ratio of MORB, symptomatic of depletion, is expected to be a long-standing feature of the upper mantle. In Fig. 1, the response of the $\bar{\kappa}_{\text{Pb}}$ parameter (and $^{208}\text{Pb}^*/^{206}\text{Pb}^*$) to recent U-Th-Pb fractionations is examined in a simple two-stage model reducing the Th/U ratio from 3.9 to 2.5 at various times in the past. For a range of possible μ values ($^{238}\text{U}/^{204}\text{Pb}$), the Pb now sampled by MORB could not have been in an environment with $\text{Th}/\text{U} = 2.5$ for more than 600 ± 200 Myr.

The simplest explanation for this observation is that the residence time ($\bar{t}_{\text{Pb}}$) of Pb in the upper mantle is short (~ 600 Myr), and, therefore, 'buffered' by the introduction of Pb into this portion of the mantle from another reservoir possessing an approximately bulk Earth $\text{Th}/\text{U} = 3.9$. Such an effect is easier

to sustain if the concentration of Pb in the upper mantle is very low compared with the introduced material. The steady-state residence time ($\bar{t}_i$) for an element (i) is given by $\bar{t}_i = [c_i/c_0]/\dot{m}_i$, where c_i is the steady-state concentration in, and c_0 that of, material added to the reservoir at a mass flux of $\dot{m}_i$ relative to the total mass of the reservoir, so that $\bar{t}_{\text{Th}}/\bar{t}_{\text{U}} = [c_{\text{Th}}/c_0]_{\text{Th}}/[c_{\text{U}}/c_0]_{\text{U}}$. For a steady-state Th/U ratio ($[c_{\text{Th}}]_{\text{Th}}/[c_{\text{U}}]_{\text{U}}$) of 2.5 and a bulk Earth value of 3.9 for the input ratio ($[c_0]_{\text{Th}}/[c_0]_{\text{U}}$), $\bar{t}_{\text{Th}}/\bar{t}_{\text{U}} = 0.64$. Similarly, if it is assumed, for example, that the $^{238}\text{U}/^{204}\text{Pb}$ ratio of the steady-state upper mantle (μ_1) is 6.0, that of the input material (μ_0) is 8.0 and fixing $\bar{t}_{\text{Pb}} = 600$ Myr, then it follows that $\bar{t}_{\text{U}} = 450$ and $\bar{t}_{\text{Th}} = 290$ Myr. The Pb isotope compositions and Th/U ratios can be readily understood in terms of short residence times for these three elements in the upper mantle, but with $\bar{t}_{\text{Th}} < \bar{t}_{\text{U}} < \bar{t}_{\text{Pb}}$ in the same order as their respective D_i .

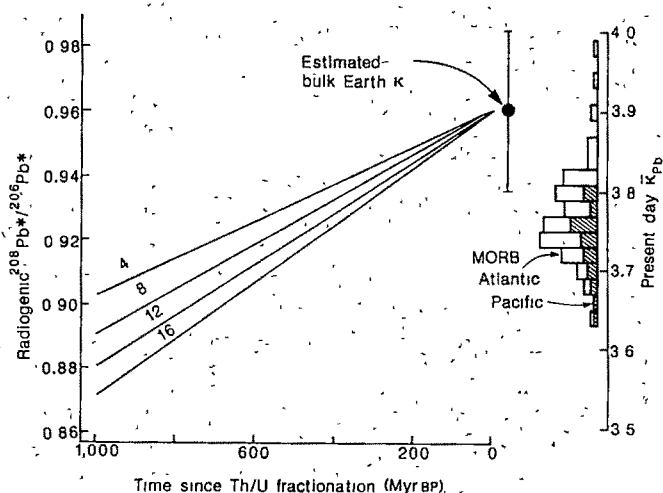


Fig. 1 Evolution of radiogenic $^{208}\text{Pb}^*$ and $^{206}\text{Pb}^*$ with time in a system with $\text{Th}/\text{U} = 2.5$ assuming a starting time-integrated Th/U ($\bar{\kappa}_{\text{Pb}}$) of 3.9. Lines are constructed for $^{238}\text{U}/^{204}\text{Pb}$ (μ) values ranging from 4 to 16. Comparison with the observed range of $\bar{\kappa}_{\text{Pb}}$ values (and $^{208}\text{Pb}^*/^{206}\text{Pb}^*$) of mid-ocean ridge basalts (MORB) indicates that they have spent 600 ± 200 Myr in a low Th/U environment. $\bar{\kappa}_{\text{Pb}}$ is defined in Table 1.

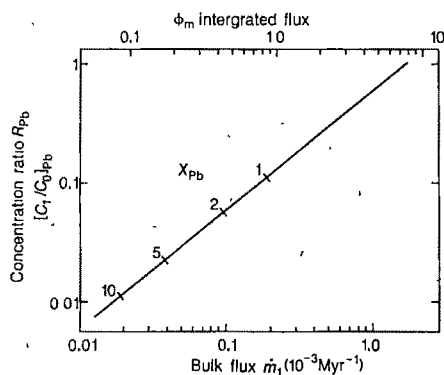


Fig. 2 The relationship between the steady-state bulk entrainment flux into the upper mantle and the degree of Pb depletion. ϕ_m is the integrated bulk flux over 4,550 Myr assuming a constant bulk flux m_1 , and are both expressed as a fraction of the upper mantle mass. c_1 is the steady-state upper mantle concentration and c_0 that of the entrained material, so that $[c_1/c_0]_{Pb}$ is the Pb depletion factor for the upper mantle. Some restriction on R_{Pb} and hence m_1 and ϕ_m arise from the Pb content of MORB. A value of $X_{Pb} = 1$ corresponds to the R_{Pb} ratio inferred to provide 400 p.p.b. Pb in MORB, assuming that major and trace elements are contributed to MORB from equal mantle source volumes and taking $[c_0]_{Pb}$ as that in the bulk silicate Earth⁵⁸. It is possible that Pb is contributed from a volume X_{Pb} times larger than that giving rise to the major elements in the oceanic crust. Acceptable solutions are likely to be restricted to $1 < X_{Pb} < 3$, corresponding to $0.25 < \phi_m < 0.5$.

Buffering and entrainment

The short residence time implied for Pb necessitates the Pb in the mantle region now sampled in MORB to have been introduced relatively recently from some other reservoir and therefore have a similar Pb isotope signature. Candidates for this external reservoir must be some portion of the continental lithosphere or a lower-mantle region which becomes entrained at its boundary with the convecting upper mantle. The first possibility is the upper continental crust, which is a substantial reservoir for Pb and either directly¹⁹, or as a component of pelagic sediment, might be injected into the mantle. Crustal Pb has a higher $^{207}\text{Pb}/^{204}\text{Pb}$ ratio than MORB that has been used to identify subducted sedimentary contributions to island-arc volcanic suites²⁰. As this elevated $^{207}\text{Pb}/^{204}\text{Pb}$ ratio is predominantly the result of ancient U/Pb fractionation recorded by the decay of now largely extinct ^{235}U , this difference indicates that the bulk of MORB Pb does not come from this source. Moreover, this situation is likely to have pertained throughout the time required to generate such a $^{207}\text{Pb}/^{204}\text{Pb}$ shift ($>10^9$ yr). An upper crustal source, and thus suggestions of substantial recycling of crustal Pb (and presumably other highly incompatible elements) back into the mantle^{21,22} are largely eliminated, although early recycling cannot be excluded. Furthermore, the subduction zone environment apparently efficiently filters continental-derived Pb, and probably also other constituents.

The second reservoir that might feasibly buffer upper mantle Pb is the mantle part of the continental lithosphere²³. This possibility may be best considered with respect to the magnitude of the bulk flux into the upper mantle that is required when integrated over a substantial portion of Earth history; a quantity that is minimized if the concentration of Pb in the upper mantle is very small compared with that in the bulk material added to the reservoir. This 'trade-off' is investigated in Fig. 2, where the concentration ratio R_i is the steady-state concentration of Pb in the upper mantle expressed as a remaining fraction of the input concentration $[c_1/c_0]_{Pb}$ for $t_{Pb} = 600$ Myr, and the total integrated bulk flux (over 4,550 Myr) into the reservoir (ϕ_m) is relative to the mass of the upper mantle.

Some limit on the Pb concentration in the upper mantle may be fixed from a consideration of that in MORB. Assuming that MORB represent a 10–20% partial melt followed by ~40% fractional crystallization, then for an average erupted value of

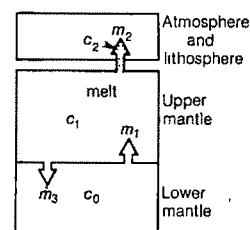


Fig. 3 A simple box model involving limited bulk entrainment at the interface between an upper and lower mantle region which are otherwise convectively isolated. It is assumed that the mass of the upper mantle box has remained constant over Earth's history, although models involving an increase in this mass and no return flux to the lower mantle box are equally viable. The concentrations of Th, U and Pb in the upper mantle box are assumed to be at steady state and supported by entrainment from the lower, minimally depleted box. The complex fractionation and removal of these elements into the continental lithosphere plus atmosphere is simply described by the single-stage extraction of partial melt as indicated. The differential partitioning of elements into the melt at this point results in variable residence times of elements in the upper mantle box and the steady-state element ratios (such as Th/U) to be different from the ratios in the material entrained at the lower boundary. However, at steady state the element ratios in input and output are equal.

400 parts per 10^9 Pb (refs 17, 24, 25), the source would contain around 20–30 parts per 10^9 . This value may be reduced by a factor X_{Pb} if the oceanic crust samples Pb from a volume that is X_{Pb} larger than from which it extracts major elements^{26,27}. This limit can now be compared with an estimate of the Pb available in the mantle part of the continental lithosphere which probably constitutes $\leq 10\%$ of the upper mantle. Comparatively Pb-rich ultramafic xenoliths from this region contain ~100 parts per 10^9 Pb (ref. 28), only a factor of 10 higher than this upper mantle value (that is, $[c_0/c_1]_{Pb} = 10$). These considerations, therefore, lead to the conclusion that the lithosphere mantle must exchange completely with the convecting upper mantle every few hundred million years to buffer upper mantle Pb, and would have a comparable age. This is, however, inconsistent with the longevity ($>10^9$ yr) of the lithosphere mantle outside the main convective flow documented by the isotope systematics of xenoliths²⁸ and silicate inclusions in diamonds²⁹. Although this does not preclude the possibility that either the mantle or crustal portions of the continental lithosphere make a contribution, they seem not to be the predominant source.

The only viable source remaining for the bulk of this Pb seems to be the lower mantle, which could buffer upper-mantle Pb for the whole of Earth's history without invoking a Pb content exceeding the bulk Earth value (Fig. 2) by virtue of its large mass. If normal ridges strip a much larger volume of mantle of incompatible elements than is indicated by the volume of oceanic crust alone^{26,27} then the estimated concentration of Pb in the upper mantle would be lowered proportionally, and similarly the flux across the boundary required to sustain this Pb. For instance, if this additional volume amounted to between 1 and 3 times that required to support the major elements in the oceanic crust ($1 < X_{Pb} < 3$), it would follow from Fig. 2 that the upper mantle would have been exchanged between a quarter and half by mass over geological time with the lower reservoir. This value is more than a factor of 10 greater than proposed by Olson³⁰ for the exchange across this boundary from scaled convection experiments.

Steady-state upper mantle

The concept of a steady-state upper mantle requires that the input and output fluxes are balanced. This has been used previously⁶ for ^3He , on the assumption that the flux into the oceans from ridges will balance an entrainment flux into the upper mantle from below. The suggested behaviour of Pb implies that, in addition to the rare gases, other highly incompatible elements in the upper mantle may also have reached steady-state con-

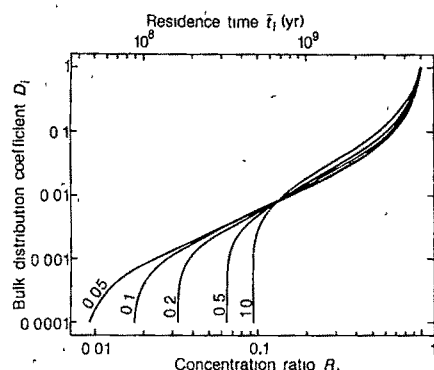


Fig. 4 Comparison of bulk distribution coefficient (D_i) and residence time ($\bar{t}_i$) in the upper mantle portion of the model depicted in Fig. 3. The ratio of steady state to input concentration (R_i) is related to the residence time by $\bar{t}_i = R_i/\dot{m}_1$ (see text). Curves are shown for various melt fractions (F , %) corresponding to the output material, but fixing $\dot{m}_1 = 0.0002 \text{ Myr}^{-1}$, $D_{pb} = 0.007$ and $\bar{t}_{pb} = 600 \text{ Myr}$. To fractionate elements of low D_i , such as Th and U, F must be small and of the order of D_i . Where $\bar{t}_i$ approaches the age of the Earth, elements will not have achieved a steady state. Elements of lowest D_i may be present in the steady state in proportions similar or identical to the input material depending on F .

centrations. Some of the ramifications of buffering by entrainment of lower mantle material are best explored within the context of a very simple model (Fig. 3) which uses several simplifying assumptions: (1) material from the lower mantle enters the upper mantle by bulk entrainment at their mutual interface without element fractionation; (2) incompatible elements in the upper mantle are transported into the overlying lithosphere and atmosphere which, as a first step, involves the generation of a small fraction of partial melt, causing element fractionations that can be modelled as Rayleigh melting; (3) the mass of the upper mantle has remained constant, with a return flux to the lower mantle during entrainment; (4) the lower mantle can be treated as an infinite reservoir with primordial mantle (bulk Earth minus core) trace-element abundances; (5) the system has reached a steady state. Note, however, that scenarios involving a variable upper mantle mass with time¹⁻⁴ have not been explored here but are equally compatible with the geochemical evidence.

The steady-state concentration [c_1], of an element of interest in the upper mantle compared with its input value [c_0], can be shown to be

$$R_i = [c_1/c_0] = 1/[1 + (\dot{m}_2/\dot{m}_1)(f_i - 1)]$$

and its residence time is

$$\bar{t}_i = R_i/\dot{m}_1$$

where $\dot{m}_1$ and $\dot{m}_2$ are the bulk mass fluxes shown in Fig. 3 expressed relative to the total mass of the upper mantle. f_i is an enrichment factor relating [c_2], to [c_1], during partial fusion³¹.

$$f_i = [c_2/c_1] = \{1 - [1 - F]^{1/D_i}\}/F$$

where D_i is the bulk distribution coefficient during melting and F is the melt fraction involved. The melt fraction F describes in a simple manner the complex extraction of elements from the mantle into the continental lithosphere. To produce the observed element fractionations F must be $\approx D_i$. An important parameter for the upper mantle is the integrated bulk flux across its lower boundary over geological time (ϕ_m). This is readily obtainable, as $\dot{m}_1$ in the steady state is $R_i/\bar{t}_i$ which, using $R_{pb} = 0.06$ (from Fig. 2) and $\bar{t}_{pb} = 600 \text{ Myr}$, implies $\dot{m}_1 \approx 0.0001 \text{ Myr}^{-1}$, or roughly half of the mass of the upper mantle has exchanged with the lower over geological time ($\phi_m \approx 0.5$). This value will be smaller if R_{pb} is smaller (for instance, if $X_{pb} > 1$).

A processing timescale (T_p), defined as the time taken to extract

partial melt once from the upper mantle mass, is given by

$$T_p = F/\dot{m}_2 = f_i \bar{t}_i / \{1 - R_i\}$$

which is also the residence time of a perfectly incompatible element ($D_i = 0$). T_p is related to the mixing time (T_m) of the upper mantle down to a scale sampled by a basalt, but not in a straightforward manner, and is estimated at $< 600 \text{ Myr}$ as $T_m < T_p \leq \bar{t}_{pb}$. An exchange timescale (T_e) can also be defined, and is the time taken to exchange the upper mantle mass once, which is simply $1/\dot{m}_1$.

The behaviour of the system is illustrated in Fig. 4, where the concentration ratio R_i and the residence time $\bar{t}_i$ are shown as a function of D_i for various values of F , fixing $\dot{m}_1 = 0.0002 \text{ Myr}^{-1}$, $\bar{t}_{pb} = 600 \text{ Myr}$ and $D_{pb} = 0.007$. The D_i/F ratio divides elements into three groups with contrasting behaviour. First, for $D_i/F \gg 1$, $\bar{t}_i >$ the age of the Earth and so a steady-state R_i does not pertain; second, for $D_i/F = 1$, $\bar{t}_i$ and R_i are at steady state and depend strongly on D_i but not F ; and third, for $D_i/F < 1$, constant $\bar{t}_i \approx T_p$ and $R_i \approx T_p \dot{m}_1$ (independent of D_i) result from the supply of material being rate limiting. For the time-dependent (non-steady state) case it can be shown that the concentration ratio decays exponentially to the steady-state value R_i in a characteristic time equal to $\bar{t}_i$.

Consideration of this simple model suggests that the more compatible elements ($D_i > 0.1$), for example, the heavy rare-earth elements (HREE), are unlikely to have reached steady-state concentrations in the upper mantle. Furthermore, the highly incompatible elements may be uniformly extracted over the upper mantle processing time (T_p) consistent with the observation that the abundance ratios of Cs, Rb and Ba (all with $D_i < 0.001$) are nearly constant in MORB³².

For those elements at steady state, input and output fluxes are necessarily equal, for example, the Th/U ratio of both the input and output materials should be 3.9 even though the steady-state ratio is 2.5. The material that is extracted from the upper mantle to reside ultimately in the continental lithosphere must be characterized by a Th/U ratio of 3.9 that should, in principle, identify the egress route. The Th/U ratios measured in MORB glasses¹⁵ are mostly < 3.0 , and tholeiitic series basalts from oceanic island arcs are commonly even lower³³; the fractionation of Th/U required does not seem to occur in either of these environments. This is analogous to the problem of the site of the $\sim 40\%$ reduction in the Sm/Nd ratio that accompanies the production of continental crust and should be addressed in any model of mantle chemistry, whether steady state or otherwise. Only calc-alkaline series island arc volcanics have Th/U and Sm/Nd ratios³³ in the range of continental crustal materials, and the problem of Th-U and rare-earth fractionation may be linked to their genesis. The present production rate of oceanic island arc volcanics³⁴ and their abundances of highly incompatible elements³³ appear comparable with that required to balance the fluxes of these elements into the upper mantle in the steady state.

Mantle chemistry and convection

The isotope compositions and abundances of the incompatible trace elements in mantle-derived materials have been exploited to constrain theories of basalt petrogenesis, delimit the major geochemical reservoirs and their interactions and appraise the possibilities of layered versus whole-mantle convection. The observation that those trace elements with particularly small distribution coefficients ($D_i < 1$) have $\bar{t}_i \approx 600 \text{ Myr}$, and that $\bar{t}_i$ varies with D_i , has important implications for the interpretation of element and isotope abundances in mantle-derived melts.

Correlations between the radiogenic isotopes of Sr, Nd, Hf, Pb and the rare gases have been a focal point of mantle geochemistry. It is now clear that only in those instances where the residence times of the isotopes of interest are the same (such as for two of the three Pb isotope systems) is their relationship likely to be straightforward. Where $\bar{t}_i$ values differ, as between He and Sr, a large degree of decoupling of their isotope compositions is to be expected, and this is exactly what is observed³⁵.

The significance of the well-documented correlations between Pb and Sr isotopes in MORB^{24,36}, and the Nd and Sr isotopes in oceanic basalts as a whole³⁷, should be re-evaluated in view of the available constraints on their residence times.

The relationships between D_i and steady-state concentration require that the layering in the mantle, as reflected in the concentration gradients across the boundary, would be most evident for those elements with the lowest D_i (such as Pb, Th, U, K, He and Ar) and progressively less perceptible as D_i increases to 1.0, whereas the opposite would be true for isotope gradients. Furthermore, that the lower mantle reservoir is mainly responsible for the supply of short t_i elements to the upper mantle must imply that they have been retained within this layer for the whole of Earth's history. The observation that most MORB have Pb isotopic compositions not far removed from that implied for a simple closed system over the age of the Earth (the geochron) is easily explained on this basis.

The small dispersion of κ_{Th} in MORB of 2.5 ± 0.2 (Table 1) when compared with the assumed bulk Earth input value of 3.9 indicates that the introduced Th has become exceedingly well-mixed into the upper mantle by the time that such Th is sampled at spreading ridge axes. The time required to mix the length-scale of input material down to the scale of sampling at ridges is, therefore, less than the residence time of Th in the system, that is a few hundred million years, and agrees with estimates from convection experiments in a layered mantle^{38,39}. Moreover, the islands such as Hawaii and Iceland that are considered, on the basis of high $^3He/^4He$ ratios, to lie over deep seated plume structures in the mantle have $\kappa_{Th} = 3.0 \pm 0.2$ indicating that entrained material is not exclusively sampled at these islands, but a considerable portion of depleted mantle is also included.

The implied short t_i values for the heat-producing elements K, Th and U has important consequences for the early thermal history of the Earth. It can be expected that these elements were rapidly concentrated, if not stabilized, in the outermost portion of the Earth (like the Moon) within the first few hundred million years of the planet's history; a significant fraction of the Earth's radiogenic heat production would, as a result, have been highly localized from earliest times. This partitioning of highly incompatible elements may have been unrelated to the formation of continental crust, a phenomenon that essentially involves major elements, and the fractionation history of Th/Pb and U/Pb recorded by the isotope structure in the continents may conceivably predate the formation of continental crust *per se*. In the case of the Moon, the large concentrations of incompatible elements in components of the lunar crust⁴⁰ do not necessarily imply that there was accompanying large-scale major element differentiation of the interior.

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Diversity and structure of genes of the α family of mouse T-cell antigen receptor

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We have analysed 19 complementary DNA clones encoding the α -chain of the T-cell antigen receptor derived from thymic transcripts, and find that 15 of them contain partial or complete variable (V_α) genes. Seven of these genes cross-hybridize to over 40 germline V_α gene segments in Southern blot analyses. Of the 19 joining (J_α) sequences examined, 18 seem to be encoded by distinct gene segments, hence the repertoire of J_α gene segments is much larger than those of the immunoglobulin or T-cell receptor β -chain gene families. We suggest that the variable domains of immunoglobulins and T-cell antigen receptors are similar in structure.

THE antigen-specific receptors on T cells, like the immunoglobulin molecules used as antigen-specific receptors on B cells, can recognize and bind an almost unlimited range of antigenic determinants. T-cell antigen recognition differs from immunoglobulin antigen recognition in that it occurs only in the context of another cell-surface protein encoded by the major histocompatibility complex (MHC), a property termed MHC restriction (for review, see ref. 1). This unique property raises the question of whether the structures of T-cell antigen receptors fundamentally differ from those of immunoglobulins.

The T-cell receptor is a disulphide-bridged heterodimer composed of α - and β -chains, each divided into variable (V) and constant (C) regions²⁻⁴. Studies of the structure and organization of β -chain gene elements have revealed that the V region of the β -chain, like that of the immunoglobulin heavy (H) chain, is encoded by three separate germline gene segments, V_β , diversity (D_β) and joining (J_β), that rearrange during T-cell differentiation to form a functional V_β gene^{5,6}. There are two closely linked C_β genes, $C_\beta 1$ and $C_\beta 2$, each associated with a cluster of six functional J_β gene segments^{5,7,8}. Each J_β cluster has one D_β gene segment located ~500-700 nucleotides upstream^{9,10}.

T and B cells use several mechanisms to generate diversity in the antigen-binding V region: (1) germline diversity. Both T and B cells use many different germline V, D and J gene segments to form a V gene. (2) Combinatorial joining. Each D gene segment seems capable of joining to any downstream J gene segment, and any V may join to any D-J combination^{11,12}. (3) Somatic mutation. The V_β genes share two somatic mutational mechanisms with their immunoglobulin counterparts. First, there is flexibility in the sites at which the gene segments are joined and this generates diversity in the junctional regions^{9,10}. Second, random nucleotides may be added to either side of the D gene segments during its joining to the V and J gene segments^{9,10}, a mechanism denoted N-region diversification¹³. Immunoglobulin V-region genes may undergo somatic hypermutation at a late stage of B-cell development, a phenomenon that has not been seen in most V_β genes^{5,11,12,14}.

The V_β gene segments differ strikingly from their immunoglobulin κ and H-chain homologues in subfamily organization. A subfamily is defined as those V gene segments that are 75% or more similar to one another at the nucleic acid level and hence cross-hybridize in Southern blots under stringent hybridization conditions¹⁵. The immunoglobulin V subfamilies are composed of 4-50 or more members¹⁵⁻¹⁷. In contrast, the known mouse V_β gene segments represent eight subfamilies, six with only one member, one with two members, and one with three members^{11,12}.

Recently, mouse cDNA clones of α -chain transcripts have been described^{18,19}. The α -chains seem to consist of V, J and C regions. Here we present the sequences of 19 additional α -cDNA clones. Analyses of these 21 α -cDNA sequences reveal that: (1) the V_α gene segments are derived from at least 10 subfamilies

each containing 1-10 members; (2) there are many J_α gene segments; (3) the diversification mechanisms for V_α genes are similar to those seen for β -chain and immunoglobulin genes; and (4) the general structure of the α -polypeptides resembles that of immunoglobulin and β -chains in primary and secondary structural characteristics.

Sequences of α -cDNA clones

Nineteen cDNA clones were isolated from a thymic library of inbred C57BL/Ka mice¹¹ using a C_α probe²⁰. The cDNA inserts were subcloned into the M13mp18 bacteriophage vector and the sequences determined using the specific-primer-directed dideoxynucleotide sequencing technique (Fig. 1)²¹. Four of the 19 α -cDNA clones (TA38, TA91, TA45 and TA20) do not have a recognizable V_α gene segment (see below); 17 contain J_α sequences; and 15 contain complete or partial V_α sequences (Figs 1, 2). The α -cDNA sequences in Fig. 1 are divided into leader, V, J and C regions according to their similarities to the V and J segments of immunoglobulins and β -chains. The V_α segment is ~90 residues long and has a leader of 19-22 amino acids (Fig. 1). The N-terminal amino acid of the mature V_α gene product is presumed to be 22 or 23 residues from the first conserved half-cysteine residue according to the general rules that have been deduced for determining the leader peptide cleavage site²². The 3' ends of the V_α sequences have been provisionally designated as the same length as that of a single germline gene segment, $V_\alpha 5H$, a member of the $V_\alpha 1$ subfamily, analysed by Winoto *et al.* in the accompanying paper²⁰. The 5' and 3' ends of four J sequences have been determined by sequence analysis of four germline J_α gene segments (TA84, TA19, TA65 and TA80)²⁰. The boundaries of the remaining J_α sequences were provisionally assigned by homology (see Fig. 1 legend). There are nucleotides present between the V_α and J_α sequences that might arise from various different sources (see ref. 20).

Subfamilies of V_α gene segments

The amino-acid sequences of the 15 V_α segments were aligned with one another and with two additional V_α segments from the literature, TT11 (ref. 18) and pHDS58 (ref. 19) (Fig. 2). The V_α segments can be divided into 10 different subfamilies, members of each subfamily being 75% or more homologous with one another at the DNA level. The V_α segments from different subfamilies are 20-54% similar to one another at the protein level (Table 1). This range of homologies is similar to that observed between the V_β and immunoglobulin V_H subfamilies¹¹. We have denoted these subfamilies as $V_\alpha 1$ to $V_\alpha 10$ (Fig. 2). Five of these subfamilies ($V_\alpha 1-5$) are represented by two members and five by only one.

To estimate the number of germline V_α gene segments in each subfamily, Southern blot analyses were performed on liver DNA from four different inbred strains of mice using V_α -containing

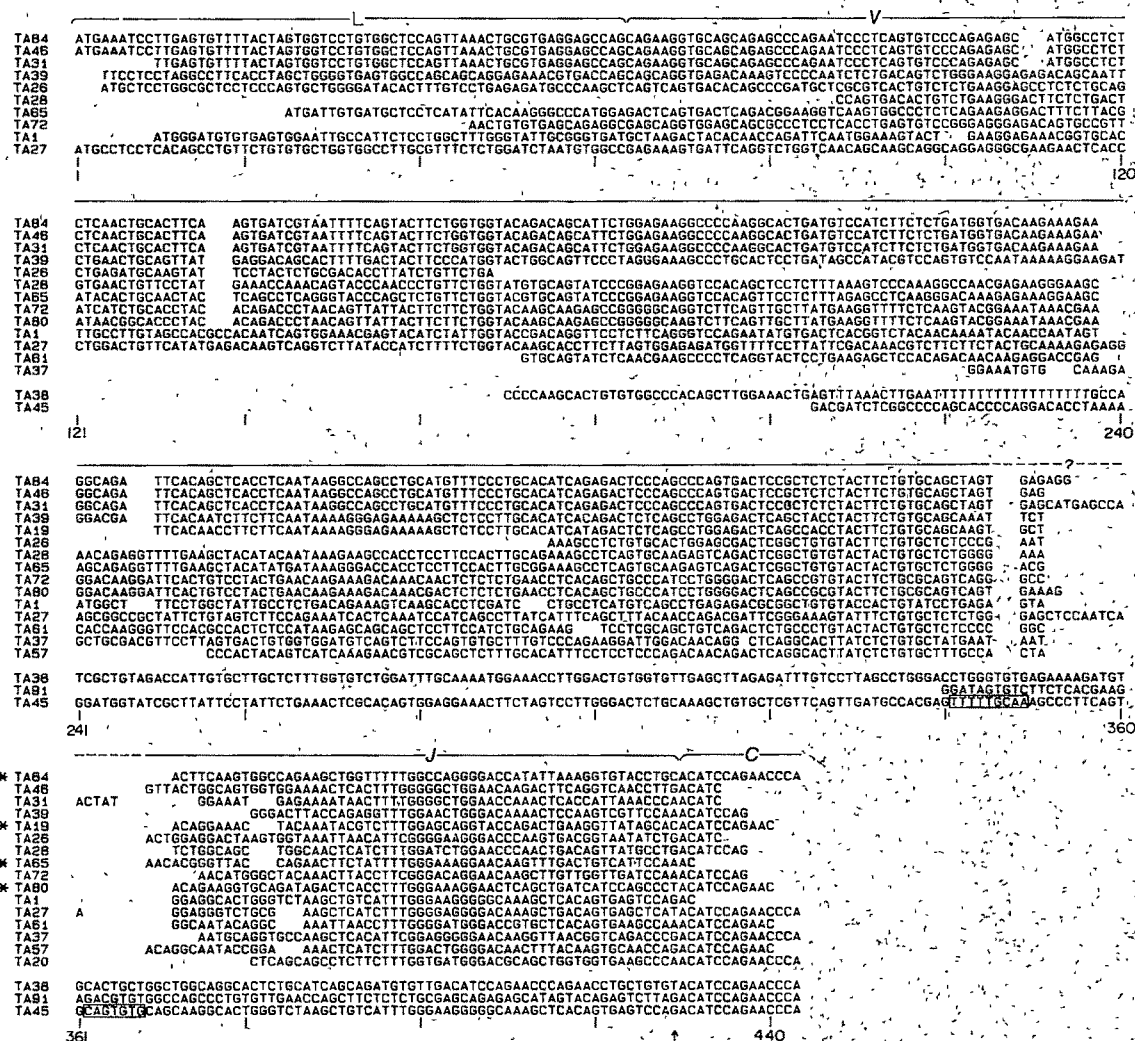


Fig. 1 Nucleotide sequences of 19 α -cDNA clones derived from the thymus. The leader (L), V, J and C regions are indicated. Clones TA38, TA91 and TA45 do not contain V_{α} sequences. The dotted lines between the V and J regions indicate junctional diversity arising from any one of several mechanisms (see ref. 20). The 3' ends of the V_{α} gene segments were arbitrarily chosen to be identical with the end of the germline $V_{\alpha}5H$ gene segment²⁰. The 5' ends of the J_{α} gene segments were determined by comparison with the corresponding germline J_{α} gene segments (indicated by asterisks)²⁰ or by arbitrarily assuming that the J_{α} gene segment begins one codon after the end of the V_{α} gene segment. Assignments of the 5' ends of the J_{α} gene segments for the TA31 and TA27 cDNA clones are complicated by the presence of frameshift mutations at the junctional regions. Gaps are inserted to maximize similarity. The arrow at base 429 indicates diversity at the end of the J_{α} gene segment (see text). In clone TA45, the recognition sequences for DNA rearrangement are boxed. A 500-bp *Sau3A1* restriction fragment containing the C_{α} gene was used as a ³²P-labelled, nick-translated probe³⁷ to screen a λ gt10 cDNA library constructed from C57BL/Ka thymus messenger RNA (ref. 38, modified by Barth *et al.*¹¹). The cDNA inserts from C_{α} -containing clones were subcloned into the M13mp18 vector³⁹. The subclones were sequenced using the specific-primer-directed dideoxynucleotide chain termination method²¹. Synthetic oligonucleotide probes complementary to both strands of the C_{α} gene were used to select inserts of both orientations.

Table 1 Similarity of V_{α} sequences

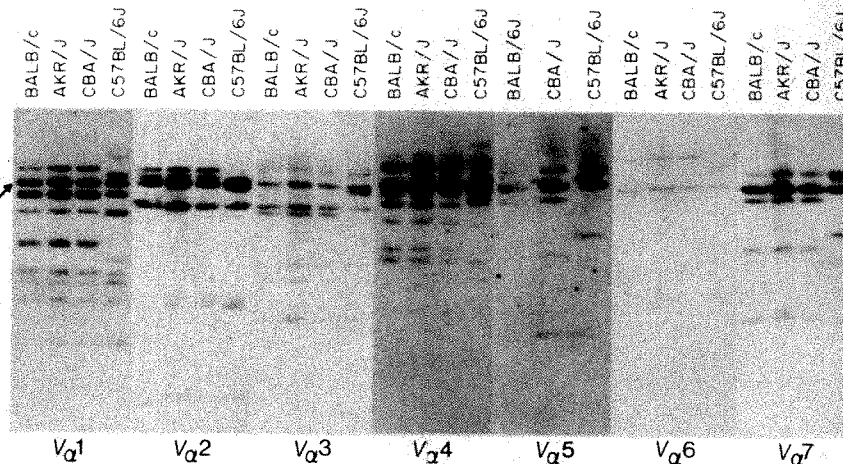
	TT11	TA84	TA39	TA19	pHDS58	TA26	TA28	TA65	TA72	TA80	TA1	TA27	TA61	TA37	TA57
TT11	—	79	51	56	33	40	31	30	43	42	20	24	38	24	40
TA84	87	—	54	65	31	35	32	31	44	42	22	28	40	22	37
TA39	61	62	—	91	33	38	35	27	44	43	25	33	29	24	43
TA19	66	69	96	—	38	41	34	31	53	56	23	44	35	23	43
pHDS58	47	44	42	46	—	96	52	50	38	37	30	27	46	26	23
TA26	48	42	44	43	96	—	60	56	38	35	35	34	69	25	47
TA28	41	43	45	46	58	63	—	70	36	33	27	26	55	18	33
TA65	42	42	40	44	60	66	83	—	30	29	26	27	54	18	33
TA72	58	60	58	67	49	53	44	44	—	90	25	34	31	16	27
TA80	53	56	56	68	47	49	42	42	95	—	20	33	30	16	30
TA1	37	36	40	41	43	48	41	40	41	40	—	22	24	19	17
TA27	40	42	43	48	40	47	43	42	47	46	37	—	25	21	40
TA61	49	52	46	51	55	69	63	62	50	49	37	39	—	22	41
TA37	36	34	37	39	40	34	28	31	30	32	35	39	29	—	31
TA57	47	41	48	49	42	53	44	41	47	49	40	41	52	37	—

Numbers above the diagonal designate percentage similarity of protein sequences; those below the diagonal compare nucleotide sequences.

	0	20	40	60	80	94	
TT11	GGNVGGSPESLIVPEGARTSLNCTF	SDSASQYFWYRQHSKAPKALMSIFSNGEKEE	GR	FTIHLNKASLHFSLHSDSPQSALYLCAVT			
TA84	K.....S.....S.....MA.....S.....	RNF.....EG.....D.....D.....K.....		A.....V.....F.....AS.....			V _α 1
TA46	S.....S.....MA.....S.....RNF.....	EG.....D.....D.....K.....		A.....V.....F.....AS.....			
TA31	K.....S.....S.....MA.....S.....RNF.....	EG.....D.....D.....K.....		A.....V.....F.....AS.....			
TA39	Q.....R.....Q.....T.....W.....ETAI.....SY	E.....TFD.....P.....W.....FPRES.....AL.....IA.....RPVSN.....K.....D.....		FF.....REKKL.....I.....G.....T.....F.....AN.....			V _α 2
TA19				TFF.....REKKL.....I.....G.....T.....F.....AN.....			
pHDS58	S.....T.....PDARVT.....S.....SLQ.....R.....KY.....Y.....TP.....LF.....V.....YPRQGLQL.....LKY.....Y.....GDPVGVGVNG.....EAEFS.....SNSS.....H.....RKASVHW.....V.....F.....LP.....			KASVHW.....V.....F.....LP.....			V _α 3
TA26	S.....T.....PDARVT.....S.....SLQ.....R.....KY.....Y.....TP.....LF.....V.....YPRQGLQL.....LKY.....Y.....GDPVGVGVNG.....EAEFS.....SNSS.....H.....RKASVHW.....V.....F.....LP.....			KASVHW.....V.....F.....LP.....			
TA28	PVTLS.....TSLTV.....SY.....ETKGYPTLF.....V.....YP.....EG.....QL.....FKVPKAN.....GS.....N.....G.....EATY.....EATS.....H.....GKASV.....E.....V.....Y.....LG.....			KASVHW.....V.....F.....LP.....			V _α 4
TA65	DS.....T.....TEGVALS.....EDFLTIH.....NY.....A.....GYPALF.....V.....YP.....EG.....QF.....FRASRDK.....GS.....S.....G.....EATYD.....GTTT.....H.....RKASV.....E.....V.....Y.....LG.....			KASVHW.....V.....F.....LP.....			
TA72	EQ.....E.....R.....PH.....S.....R.....DSAVII.....Y.....T.....PN.....Y.....F.....K.....EP.....AGLQL.....KV.....ST.....IN.....QG.....VL.....KDKQL.....NLTAAH.....G.....V.....F.....R.....			KASVHW.....V.....F.....LP.....			V _α 5
TA90	ITAPY.....T.....PN.....Y.....F.....K.....EP.....AGLQL.....KV.....ST.....IN.....QG.....VL.....KDKQL.....NLTAAH.....G.....V.....F.....R.....			KASVHW.....V.....F.....LP.....			
TA1	DAKTT.....PDSMEST.....ETVH.....P.....SHATI.....GNE.....IY.....VPLQG.....EYVTHGLQNTTNS.....MA.....LAIASDRKSST.....I.....LPHVSLR.....A.....V.....H.....ILR.....			KASVHW.....V.....F.....LP.....			V _α 6
TA27	EK.....I.....VWSTASRQ.....EELT.....D.....SYETSQVLYHLF.....V.....YLNE.....QV.....LKSSTDNKRT.....HGG.....HAT.....H.....S.....SS.....H.....QK.....S.....L.....Y.....LP.....			KASVHW.....V.....F.....LP.....			V _α 7
TA81				KASVHW.....V.....F.....LP.....			
TA37				KASVHW.....V.....F.....LP.....			V _α 8
TA57				KASVHW.....V.....F.....LP.....			V _α 9
				KASVHW.....V.....F.....LP.....			V _α 10

Fig. 2 Protein sequences of 17 V_{α} gene segments. Clone designations (left) and V_{α} subfamily designations (right) are given. Dots indicate that the sequence is identical to that of clone TT11. The blanks indicate sequence gaps introduced to maximize homology. The asterisks indicate residues that are relatively conserved in V_H , V_L , V_B and V_{α} regions.

Fig. 3 Southern blot analysis of mouse germline DNA with complete α -cDNA clones from seven V_{α} subfamilies. Liver DNA from four inbred strains of mice was isolated⁴⁰ and *Bam*HI-digested. Similar results were obtained using liver DNA digested with *Eco*RI and *Hind*III (not shown). The 9.8-kilobase C_{α} -hybridizing bands on each filter have been aligned here and are indicated by an arrow. As the DNA migration distances varied slightly from gel to gel, hybridizing bands at the same location on different filters do not necessarily contain DNA fragments of precisely the same size. Each DNA digest (10 μ g) was electrophoresed on 0.7% horizontal agarose gels and transferred to nitrocellulose⁴¹. Hybridizations were for 24 h at 37 °C in 50% formamide, 0.8 M NaCl, 0.1 M Tris pH 7.5, 5 $\times$ Denhardt's, 100 μ g ml⁻¹ denatured salmon sperm DNA, 10% dextran sulphate and 0.5 μ g ³²P-labelled nick-translated cDNA probe. Filters were washed at 68 °C with 1 $\times$ SSC containing 0.1% SDS.



*Eco*RI fragments as probes (Fig. 3). Because some of the α -cDNAs contain only a small segment of V_{α} sequence (Fig. 1), only seven of the V_{α} subfamilies were analysed. Assuming that each distinct band represents at least one separate V_{α} gene segment, the V_{α} subfamilies analysed here range in size from 1 to 10 members, and the minimum number of bands identified for all seven subfamilies is 40. This distribution is similar to that seen in the immunoglobulin κ - and H-chain gene families and contrasts with the V_{β} gene family, where six of eight subfamilies analysed contained just one member, and the total V_{β} repertoire is believed to be <21 members^{11,12}.

Three identical V_{α} sequences have been observed to be associated with three distinct J_{α} segments, a surprising result for which we can offer no explanation apart from the possibility that rearrangement and expression of this V_{α} sequence may be preferred in the thymus. The remaining 12 V_{α} sequences are all distinct from one another (Fig. 2).

Restriction length polymorphism is observed between inbred C57BL/6J mice and the three other strains of mice analysed (Fig. 3). Differences are seen with all seven V_{α} probes. In addition, some subfamilies in different mouse strains appear to differ in their V_{α} gene segment numbers; for example, compare the C57BL/6J strain with the others (Fig. 3). The immunoglobulin H- and κ -chain gene families also exhibit extensive restriction length polymorphism, whereas the V_{β} gene family shows very limited polymorphism. Variation in band number within particular subfamilies probably represents the duplication and/or deletion of V_{α} gene segments in the various inbred strains of mice. These data are consistent with the hypothesis that multi-membered V subfamilies may undergo gene expansion and contraction by homologous but unequal crossing-over (see ref. 11).

Numerous J_{α} segments

Of the 17 J_{α} sequences described in this paper and two from the literature^{18,19}, 18 different J_{α} sequences have been identified (Fig. 4). As we found only one repeat, the repertoire of J_{α} gene

segments is probably much larger than 18. We believe that each of these different sequences represents a distinct germline J_{α} gene segment as they differ considerably from one another in sequence. The J_{α} segments exhibit a range of similarity at the protein level (32–72%) comparable with that for J_{β} segments (33–75%) and somewhat lower than that seen for J_{κ} (55–85%), J_H (57–80%) and J_{λ} (70–85%) segments. The J_{α} gene segment repertoire is larger than those of the β -chain (12) or immunoglobulin (4–5) genes. The large repertoire and diversity of the J_{α} gene segments may have important implications for antigen/MHC recognition, as the J segments in immunoglobulins usually fold to form a contact point of the antigen-binding site.

Nonproductive thymic V_{α} transcripts

Nonproductive α -cDNAs may arise by joining events which place the J gene segment in an improper translational reading frame with respect to the V gene segment, by rearrangement of a pseudogene segment or by initiation of transcription 5' to a germline J gene segment or 5' to an incomplete D–J rearrangement. Of the 15 α -cDNAs that have V_{α} gene segments, 5 seem to be derived from nonproductive transcripts. Three of these may have resulted from the rearrangement of a pseudogene. One clone, TA37, has a frameshift within the V_{α} gene segment caused by a single nucleotide deletion at position 317 (Fig. 1). The TA80 clone seems to have a base substitution that has changed the cysteine residue at position 22 to alanine (Fig. 2). This cysteine residue is invariant in immunoglobulins and β -chains and is believed to be important for stabilizing the V domain structure. In addition, the V_{α} and J_{α} gene segments have joined so that the J_{α} gene segment is out of the proper translational reading frame. Thus the TA37 and TA80 clones probably represent transcripts derived from the rearrangement of pseudogenes. A third clone, TA26, has a V_{α} gene segment virtually identical to the previously published pHDS58 V_{α} gene¹⁹ apart from a large deletion in the centre of the V_{α} region encompassing 43 residues and creating a translational frame-

shift. Two base changes within the coding region do not lead to changes in amino-acid sequence from the pHDS58 V_α segment and might represent polymorphism between the inbred BALB/c and C57BL/Ka mice. The TA26 V_α gene segment may be a pseudogene that has been rearranged and transcribed (see ref. 23), or alternatively the deletion could represent a cloning artefact. Finally, the TA27 and TA31 clones also contain a V_α - J_α junction which shifts the J_α sequence out of the correct translational reading frame. Nonproductive rearrangements of this type have been reported previously for both immunoglobulin²⁴ and T-cell receptor β -chain genes (M. Malissen, unpublished data) and are often transcribed²⁵. The remaining 10 V_α -containing clones seem to represent productive or functional V_α - J_α rearrangements, although we cannot exclude the possibility that one or more of the partial V_α sequences have mutations in their unsequenced regions that would cause them to be pseudogenes.

α -cDNA clones lacking V_α

Of the 19 cDNA clones analysed, 4 did not contain V_α sequences. One clone, TA20, extends only into the 5' half of a J_α gene segment. A second, TA45, seems to be either a germline J_α transcript or a transcript from an incomplete D - J rearrangement. Both of these transcription events are known to occur in β -chain genes^{9,26}. The TA45 clone contains a J_α sequence and a 5' recognition sequence for DNA rearrangement, consisting of a conserved heptamer, a spacer sequence of 12 nucleotides and an A/T-rich nonamer. The germline V_α gene segment has the rearrangement recognition sequence with a 23-base-pair (bp) spacer²⁰. In immunoglobulin and β -chain genes, gene-segment rearrangements always occur between recognition sequences with 12- (one turn of the DNA helix) and 23-bp (two turns) spacers^{5,6,27,28}. Hence, this one-turn recognition sequence can join to the two-turn recognition sequences of the V_α genes²⁰. As the germline sequence of this J_α gene segment has not been determined, we do not know whether the recognition sequence lies to the 5' side of a D_α or a J_α gene segment. Finally, two of the cDNA clones encode complete C_α , but no J_α or V_α regions. The sequences to the 5' side of the C_α genes are not similar to each other or to the intron sequences flanking the germline C_α gene (A. Winoto, unpublished data). Presumably, these cDNA clones represent aberrant RNA splicing events.

V_α gene diversification mechanisms

The α -chain genes apply three mechanisms used by the immunoglobulin and β -chain genes for diversification.

Germline. Southern blot analyses with V_α probes and the V_α sequence analyses suggest that at least 10 different V_α subfamilies exist which encode >40 V_α gene segments. Only one V_α sequence is repeated in the sample of cDNAs analysed. A comparable analysis of 22 V_β sequences revealed 11 repeat V_β sequences and only 13 distinct V_β gene segments¹¹. Statistical analyses of thymus β -chain cDNA clones indicate that there are ≤ 21 germline V_β gene segments¹¹ and perhaps only two D_β gene segments^{11,14}. Hence the diversity of the V_α gene segment repertoire seems to be considerably larger than that of the V_β gene segments. The germline J_α repertoire seems to be quite large in that 18 out of 19 sequences sampled are different. This J_α diversity greatly increases the germline diversity at the carboxy-terminal end of the V_α region. Although provisional alignments of the different α -cDNA clones with the available germline V_α and J_α sequences reveal additional nucleotides at the V - J junctions (Fig. 1), additional germline α -sequences will have to be determined before the existence of D_α gene segments can be established (see ref. 20).

Combinatorial joining. Two examples of combinatorial joining in the α -family have been observed in the sample of α -cDNA sequences examined. First, the same V_α sequence in clones TA84, 46 and 31 has been joined to three distinct J_α gene segments (Fig. 1). Similarly, the same J_α sequence has been joined to two distinct V_α gene segments (TA28 and A. Winoto, unpublished data). Therefore, the combinatorial joining of V_α and J_α gene segments can occur in the α -family.

	1	10	20
TT11	YGGSGNKLIFGTGTLSSVKPN		
TA84	TS..Q..V..Q..I..K..YLH		
TA46	VT...G...TL..A...R..Q..NLD		
TA31	..N..E..IT...A...K..T...I...		
TA39	...TYQR...K..Q..V...		
TA19	T..N..Y..YV...A...R..K..IAH		
pHDS58	..FASA..T...S...KVI..L..Y		
TA26	T...LSG...T...E...QVT..ISD		
TA28	S...WQ...S...Q..T..M..D		
TA65	NT..Y..QNFY...K...S..T..I...		
TA72	NM..Y...T...S...L..D...		
TA80	TEGADR..T...K...Q..IIQ..Y		
TA1	...T..S...S...K..AK..T..S..D		
TA27	...A...E...K..T..SSY		
TA61	..NT...T...D...V..T...		
TA37	NA..A...T...G...R..T..R..D		
TA57	T..NT...L...T..Q..Q..D		
TA20	LSS..F...D...Q..V...		

Fig. 4 Protein sequences of J_α segments. The N- and C-terminal boundaries of these sequences have been approximated using the germline sequences of six J_α gene segments²⁰. Asterisks indicate amino acids conserved in the J_α , J_β , J_H and J_L regions. Dots indicate identity to the TT11 sequence. Gaps are inserted to maximize similarity. The boundaries of the remaining J_α regions were provisionally assigned to the second residue following the putative C-terminal end encoded by the V segment. Diversity in the C-terminal residue is generated by RNA splicing (see text).

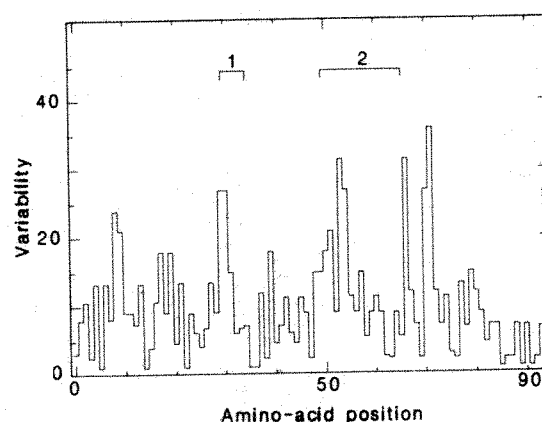


Fig. 5 Variability plot of V_α regions. Variability was determined according to the method of Wu and Kabat³¹. Nine V_α segments were analysed. The variability at each amino-acid position N is calculated as: $\text{Variability}_N = (\text{No. of different amino acids that occur at } N) / (\text{Frequency of most common amino acid at } N)$. For comparison, the first and second hypervariable regions of V_H segments are indicated.

Somatic mutation. The delineation of junctional and N-region diversification mechanisms requires a comparison between germline and rearranged V_α sequences. These mechanisms are used in the V_α gene family, as demonstrated in the accompanying paper²⁰. The fact that three V_α gene segments are identical to one another suggests that somatic hypermutation has not occurred in these α -chain genes. However, somatic hypermutation occurs late in B-cell development. Here we are analysing antigen-receptor genes at an early stage of T-lymphocyte development, possibly before somatic hypermutation. Data on the receptors of mature T cells will be necessary to determine whether somatic mutation does occur in V_α genes.

Diversification of a special type occurs at the J_α - C_α boundary. The last nucleotide in the J_α gene segments may be any of the four bases (Figs 1, 4). In the immunoglobulin and β -chain gene families the final nucleotide of the J gene segment is invariant. As RNA splicing joins the last nucleotide of the J gene segment to the first two nucleotides of the C gene, four different junctional codons are generated at the J_α - C_α junction: asparagine (AAC), histidine (CAC), aspartic acid (GAC), and tyrosine (TAC). It will be interesting to determine whether this boundary

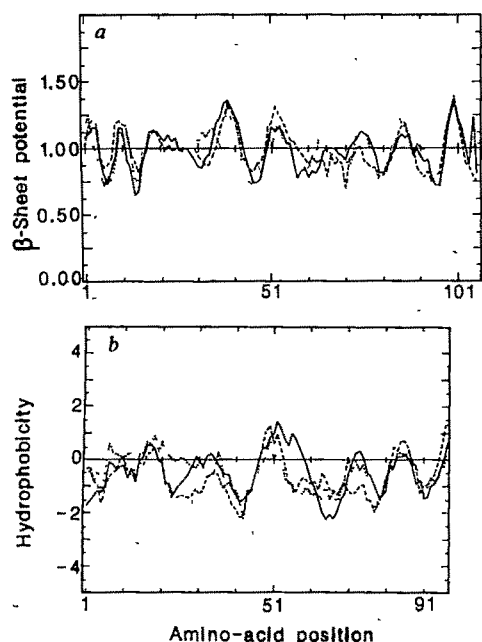


Fig. 6 Secondary structural analyses of V_{α} (solid lines), V_H (dotted lines) and V_{β} (dashed lines) regions. *a*, Plot indicating the β -pleated sheet-forming potential of V_{β} , V_H and V_{α} segments using the algorithm of Chou and Fasman^{34,35}. *b*, Plot of the relative hydrophobicity of the side chains of the amino acids constituting the V_{α} , V_{β} and V_H regions, using the algorithm of Kyte and Doolittle³³. The V_{β} sequences and V_H sequence compilations are from Barth *et al.*¹¹.

diversity has any functional significance for the T-cell antigen-binding receptor.

Structure of the V_{α} region

We have analysed the primary and secondary structures of the V_{α} regions to determine whether they resemble the V regions of β -chains and immunoglobulins.

Both the V_{α} and V_{β} regions share many conserved residues with both the V_H and variable light-chain (V_L) regions of immunoglobulins, including 14 residues in the V and 4 residues in the J regions (marked by asterisks in Figs 2, 4). An analysis of the three-dimensional structures of the V domains in several immunoglobulin molecules demonstrates that there are many highly conserved residues important for stabilization of V_L - V_H interactions and for intrachain V_L or V_H interactions^{29,30}. Most of these residues are conserved in the V_{α} and V_{β} regions.

Another approach to elucidate primary structural patterns in the T-cell receptor and immunoglobulin V regions is the variability plot of Wu and Kabat³¹; this analysis examines the distribution of variation of each residue position between members of a set of similar sequences. In immunoglobulin and β -chain V regions, two regions of relative hypervariability are noted, with

a third hypervariability³¹ region positioned at the junction of the V, D and J gene segments^{11,32}. A variability plot of the V_{α} sequences does not differ dramatically from the V_H and V_{β} variability plots (Fig. 5 and refs 11, 12). With the limited number of V_{α} sequences analysed, one must be cautious in drawing generalizations for the overall variability in this set is high. However, the regions corresponding to the two classically defined hypervariable regions (residues 30–35 and 51–60) seem to have increased variability. We note two additional areas of hypervariability (residues 8–9 and 66–71) but feel their existence is uncertain until more V_{α} -sequences are available for analysis. The junction of the V_{α} and J_{α} segments generates an additional hypervariable region (not shown).

Two further analyses have been conducted on the V_{α} sequences to assess the nature of their secondary structure. The Kyte-Doolittle hydrophobicity profile³³ plots the distribution of the relative hydrophobicities of the amino-acid side chains of a particular protein. The Chou-Fasman method^{34,35} determines the relative potential for β -pleated sheet formation. Both these analyses measure properties that are thought to reflect important secondary structural features of the protein. The Kyte-Doolittle hydrophobicity and the Chou-Fasman plots of the V_{α} , V_{β} , V_H and V_{κ} (not shown) sequences are very similar (Fig. 6). The plots represent averages of multiple sequences to minimize unusual individual variations. These data indicate that the secondary structures of the V_{α} , V_{β} , V_H and V_{κ} regions are similar to each other.

Thus, several lines of evidence suggest that general primary and secondary structural features are conserved in the V_{α} , V_{β} , V_H and V_{κ} regions: (1) they share highly conserved amino-acid residues, most of which are important for stabilization of the highly conserved immunoglobulin fold; (2) the variability plots demonstrate variability consistent with three hypervariable regions; and (3) the hydrophobicity and Chou-Fasman plots suggest similar potential for β -pleated sheet formation. These data suggest that the T-cell antigen receptor and immunoglobulin molecules have similar structure. Moreover, as immunoglobulins may also be MHC-restricted³⁶, it is unnecessary to postulate any special sites, apart from the classical antigen-binding site, for binding properties of T-cell MHC-restricted antigen receptors.

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Early *Homo erectus* skeleton from west Lake Turkana, Kenya

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The most complete early hominid skeleton ever found was discovered at Nariokotome III, west Lake Turkana, Kenya, and excavated in situ in sediments dated close to 1.6 Myr. The specimen, KNM-WT 15000, is a male Homo erectus that died at 12 ± 1 years of age, as judged by human standards, but was already 1.68 m tall. Although human-like in many respects, this specimen documents important anatomical differences between H. erectus and modern humans for the first time.

DURING the course of palaeontological exploration on the west side of Lake Turkana, Bw. Kamoya Kimeu found a small fragment of hominid frontal bone exposed on the surface at the site of Nariokotome III, on the south bank of the Nariokotome River. The approximate latitude and longitude of the site are $4^{\circ}08' \text{ N}$, $35^{\circ}54' \text{ E}$ (Fig. 1). Near the site the Plio-Pleistocene beds strike $\text{N } 7^{\circ} \text{ E}$ and dip 5° to the west. Exposures are reasonably good along the south bank of the Nariokotome where a section was measured to establish the stratigraphic position of the hominid (Fig. 2). Several tuffs occur within this section that have been correlated with tuffs elsewhere in the Turkana Basin on the basis of their chemical composition. The hominid derives from a siltstone that immediately overlies a tuff identified as a component ash of the Okote Tuff complex of the Koobi Fora Formation. The age of this tuff is $\sim 1.65 \text{ Myr}^{1,2}$. An ash that correlates with Tuff L of the Shungura Formation (Chari Tuff of the Koobi Fora Formation) dated at $1.39 \text{ Myr}^{3,4}$ lies 34 m above the hominid level. An un-named tuff dated at 1.33 Myr^4 lies 46 m above the specimen. Thus, the hominid is probably very close to 1.6 Myr in age.

The strata consist predominantly of pale yellowish-brown sandstones and siltstones and very pale yellowish-brown to medium-brown siltstones. The sandstones and siltstones are either laminated or massive. The tuff that underlies the hominid fills cracks in an underlying sandy siltstone and contains small-to-medium-scale trough crossbeds truncated at their tops and overlain by siltstone. A small lens of fine tuffaceous sand that lies $\sim 1 \text{ m}$ above the hominid level contains abundant amphioxea and amphistrongyla of freshwater sponges. A tuff that lies 6.7 m above the hominid level contains reworked molluscs at the base and a sandstone 13.8 m above the hominid is capped by an ostracod-rich layer 10 cm thick. Mammalian fossils are rare at this locality, the most abundant vertebrate fossils being parts of small and large fish. The depositional environment was evidently an alluvial plain of low relief, consistent with the fossil fauna in the section. It is likely that the plain was only slightly higher in elevation than a lake which existed nearby, such that with only minor changes in lake level, typical lacustrine forms (for example, ostracods, molluscs) could invade the area.

Twenty-five other vertebrate-bearing sites were located and collected during the 1984 season. Individual sites were labelled by the name of the ephemeral river draining their exposures and were further numbered sequentially in order of their discovery (Table 1). As at Koobi Fora, the Okote Tuff was used as a marker horizon in the subdivision of the fossiliferous succession. Fossil assemblages were retrieved from horizons a short depth below the Okote Tuff at five localities and slightly above it at eight localities (see Table 2). Appreciably fewer taxa are represented at these new localities than at equivalent places at Koobi Fora, but this may be an artefact of sample size. The distribution of identifiable species at sites on both sides of the lake, specifically the presence of *Deinotherium bozasi*, *Elephas recki ileretensis*, *Diceros bicornis*, *Metridiochoerus andrewsi* and

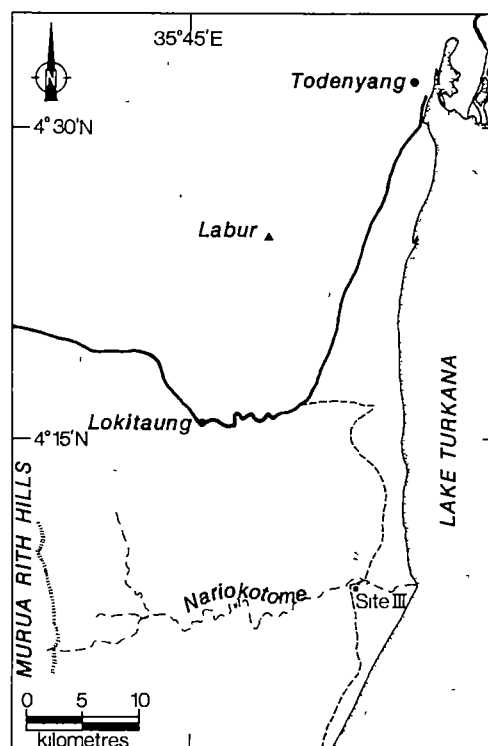


Fig. 1 Map of northern Kenya to show location of site Nariokotome III.

Gazella janenschi only at horizons below the Okote tuff and *Metridiochoerus compactus* only at horizons above this tuff, provides a measure of correlative support for geochemical analyses of the tephra. *Tragelaphus scriptus* is the only species from this part of the section on the west side of the lake that is not represented yet at Koobi Fora.

Following the initial discovery, screening and washing of surface float and pebble lag led to the recovery of most of the hominid calvaria. The facial skeleton was found just eroding out from tuffaceous sediments. Excavation of an area $\sim 5 \times 6 \text{ m}$ has led so far to the recovery of the mandible, several isolated teeth and much of the postcranial skeleton. A list of the parts found so far is given in Table 3. The site plan of the excavation (Fig. 3) shows that the skeleton was dispersed before final sedimentary burial. The bones were found in a layer of tuffaceous silt of variable thickness deposited on a more indurated, flat-lying tuffaceous sand with orange root casts. The top of the fossiliferous horizon shows many signs of bioturbation and several of the bones were found broken or lying in positions suggesting that they had been trampled by large mammals. The

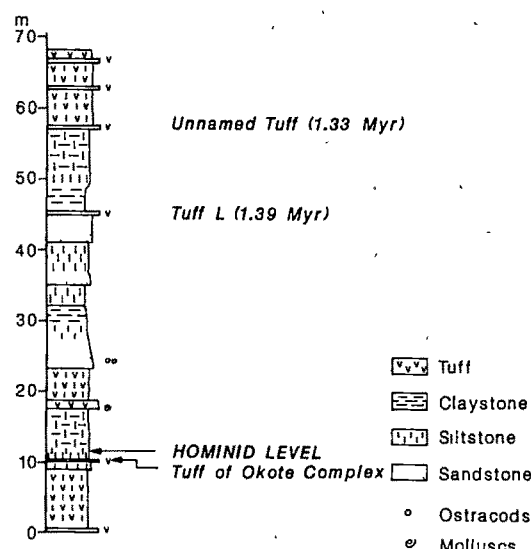


Fig. 2 Section at site Nariokotome III to show stratigraphic position of KNM-WT 15000.

general alignment of the long bones, separation of elements once in close articulation and the linear dispersal of the skeleton over at least 7 m, indicate minor water transport (see Fig. 3). Postmortem damage is seen and some parts of the bones are crushed, particularly where there is much cancellous tissue. There are no signs of carnivore or scavenger damage and the only pathology is a minor amount of alveolar resorption on the right mandibular body which took place before the right dm_2 was shed. The only other fauna found so far in the fossiliferous bed are many opercula of the swamp snail *Pila*, a few bones of the catfish *Synodontis* and two fragments of indeterminate large mammal bone, although equid and pygmy hippopotamus remains were found on the surface here.

The cranium has been assembled from about 70 pieces. The only main pieces missing are the nasals, ethmoid, lacrimals, central parts of the supraorbital tori, parts of the sphenoid and some parts of the vault, the two largest being bits of the right frontal near bregma and right parietal near lambda. The sutures were all unfused. The facial skeleton was positioned on the braincase by using the mandible. The mandible is undistorted, but the calvaria has a slight overall plastic deformation with the upper part displaced to the left. Only a preliminary cranial reconstruction has been possible so far and the capacity has not been measured. Table 4 lists the teeth found so far and their measurements. They are all close to the mean size recorded for *Homo erectus* from Zhoukoudian⁵. The M3 germs will have to be examined radiologically. Several of the teeth were excavated separately and returned to their alveoli. The alveoli for the deciduous upper canines are still visible lateral to the permanent canine alveoli, and the left upper canine was in the process of erupting. The degree of root development of the isolated teeth, the tooth wear stages and the eruption stage are equivalent to that in 12 ± 1 -year-old modern human males⁶. The canines erupted later than the M2s, and although this is not usual, it is a known human condition⁷. The dental age is matched in the postcranial skeleton where all epiphyses are unfused and the incongruity between growth-plate surfaces suggests that much further growth would have been possible. The cranium does not show strong tori, temporal or nuchal lines because it was immature at death. Despite this, its tori are thicker, its palate broader and its facial skeleton bigger and more massive than those of the presumed female cranium KNM-ER 3733 (ref. 8), which is from a roughly equivalent time horizon in the same basin. We take this to mean that there was probably considerable sexual dimorphism in the crania of early *H. erectus*. It is conceivable that this individual, had it lived to maturity, might have developed as strong and robust a cranium as that of Olduvai Hominid 9 (ref. 9).

Table 1 Fossiliferous sites west of Lake Turkana

Locality	Abbreviation	Horizon
Kaitio I	KI I	Indet.
Kaitio 11	KI 11	Indet.
Kalachoro I	KL I	Below Chari Tuff
Kalachoro II	KL II	Below Chari Tuff
Kalachoro III	KL III	Below Okote Tuff
Kalachoro IV	KL IV	Indet.
Kalachoro V	KL V	Below Chari Tuff
Kalachoro VI	KL VI	Below Okote Tuff
Kalakodo	KK	Below KBS Tuff
Kangaki	KG I	Below KBS Tuff
Kangaki II	KG II	Below KBS Tuff
Loruth Kaado I	LK I	Below Tulu Bor Tuff
Loruth Kaado II	LK II	Below Tulu Bor Tuff
Loruth Kaado III	LK III	Below Chari Tuff
Loruth Kaado IV	LK IV	Below Okote Tuff
Nachakui I	NC I	Above Chari Tuff
Nachakui II	NC II	Above Chari Tuff
Nachakui III	NC III	Indet.
Nanyangakipi	NN	Just below/above Okote Tuff
Nariokotome I	NK I	Galana Boi Beds
Nariokotome II	NK II	Below Chari Tuff
Nariokotome III	NK III	Immediately above Okote Tuff
Nariokotome IV	NK IV	Below Chari Tuff
Natoo	NT	Below Okote Tuff
Nyaena Engol I	NY I	Below Okote Tuff
Nyaena Engol II	NY I	Indet.

Indet., indeterminate horizon.

Previous *H. erectus* postcranial material has been either fragmentary, not definitely associated, disputed as to species or diseased. From Trinil, Indonesia¹⁰, there are several fragmentary and one complete (but pathological) femora. Despite the fact that it was these specimens that led to the species name, there are doubts as to whether they are *H. erectus*¹¹ with the most recent consensus being that they probably are not. Until recently the only *H. erectus* postcranial bones from China were from Zhoukoudian¹² and these were very fragmentary, with no complete lengths or articular surfaces. The reported clavicle is probably not even primate¹³. Recent newspaper accounts report an *H. erectus* cranium, innominate, ulna, three vertebrae, a rib; some hand and foot bones, a patella and a partial innominate from Yingkou, Liaoning Province, China, which are said to be 200,000 yr old^{14,15}.

H. erectus postcrania have also been found at Olduvai Gorge, Tanzania, and East Turkana, Kenya. An associated fragmentary left innominate and femoral shaft (OH 28) from Bed IV Olduvai have been described as belonging to this species¹⁶. Tibial and femoral shafts (OH 34) from Bed III Olduvai¹⁷ may belong, but are so badly eroded that their analytical value is slight. An undescribed ulna (OH 36) from Bed II Olduvai has also been attributed to this species¹³. There are many postcranial bones from East Turkana^{18,19}. Only one is definitely associated with complete enough cranial remains to be absolutely certain of attribution and, unfortunately, that individual had suffered from a disease which affected the postcranial skeleton^{19,20}. The East Turkana specimens are mainly isolated and/or fragmentary.

The finding of KNM-WT 15000 will allow not only the study of the morphology and proportions of this individual, but also allow firmer attributions and enhance the analytical value of very many other isolated or fragmentary specimens. Recognizing that the excavation has yet to be completed and that this will probably result in the recovery of more parts, a complete assessment of this individual will not yet be attempted. There are, however, some points which can confidently be made. The stature of the individual has been estimated by using regression equations developed on modern human adult males²¹ to be 1.68 m (caucasian) or 1.64 m (blacks). This individual would not have been quite that tall, because the cranium does not have the great height of modern *Homo sapiens*. Despite the young age of the individual, the length of the long bones are very close

Table 2 Faunal list

Taxon	West Turkana		East Turkana	
	KLIII, KLIV, LKIV, NY, NN	NT, LKIII, KLI, KLII, KLV, NKIII	Below Okote	Above Okote
Pisces	✓	✓	✓	✓
<i>Crocodylus</i> sp.		✓	✓	✓
<i>Euthecodon brumpti</i>		✓	✓	✓
<i>Trionyx</i> sp.	✓	✓	✓	✓
<i>Geochelone</i> sp.		✓	✓	✓
Aves	✓	✓	✓	✓
<i>Hystrix</i> sp.		✓	✓	✓
<i>Theropithecus oswaldi</i>	✓	✓	✓	✓
<i>Homo erectus</i>		✓	✓	✓
<i>Canis</i> sp.	✓	✓	✓	✓
<i>Carnivora</i> gen indet		✓	✓	✓
<i>Deinotherium bozasi</i>	✓		✓	
<i>Elephas recki ileretensis</i>	✓		✓	
<i>Elephas recki recki</i>		✓		✓
<i>Hipparion ethiopianum</i>		✓	×	×
<i>Equus koobiforensis</i>	✓	✓		
<i>Diceros bicornis</i>	✓		✓	
<i>Ceratotherium simum</i>	✓		✓	✓
<i>Metridiochoerus andrewsi</i>	✓		✓	✓
<i>Metridiochoerus modestus</i>	✓		✓	✓
<i>Metridiochoerus compactus</i>		✓	✓	✓
<i>Kolpochoerus limnetes</i>		✓	✓	✓
<i>Hexaprotodon karumensis</i>	✓	✓	✓	✓
<i>Hippopotamus gorgops</i>	✓	✓	✓	✓
<i>Hippopotamus aethiopicus</i>	✓	✓	✓	✓
<i>Aepyceros</i> sp.	✓	✓	✓	✓
<i>Megalotragus</i> sp.		✓	✓	✓
<i>Connochaetes</i> sp.	✓	×	✓	✓
<i>Damaliscus</i> sp.	✓	×	✓	✓
<i>Pelorovis</i> sp.	✓	✓	✓	✓
<i>Pelorovis olduwayensis</i>		×	✓	✓
<i>Syncerus caffer</i>	×	×	×	✓
<i>Tragelaphus strepsiceros</i>	✓	✓	✓	✓
<i>Tragelaphus scriptus</i>	✓	✓	✓	✓
<i>Menelikia lyrocera</i>		✓	✓	✓
<i>Kobus sigmoidalis</i>	✓		✓	✓
<i>Kobus kob</i>		✓	✓	✓
<i>Kobus leche</i>		?	✓	✓
<i>Antidorcas recki</i>	✓		✓	✓
<i>Gazella janenschi</i>	✓		✓	✓
<i>Gazella praethomsoni</i>	✓		✓	✓
<i>Gazella</i> sp.		✓	✓	✓

✓, Species present; ×, comparable species present; ?, stratigraphic position uncertain.

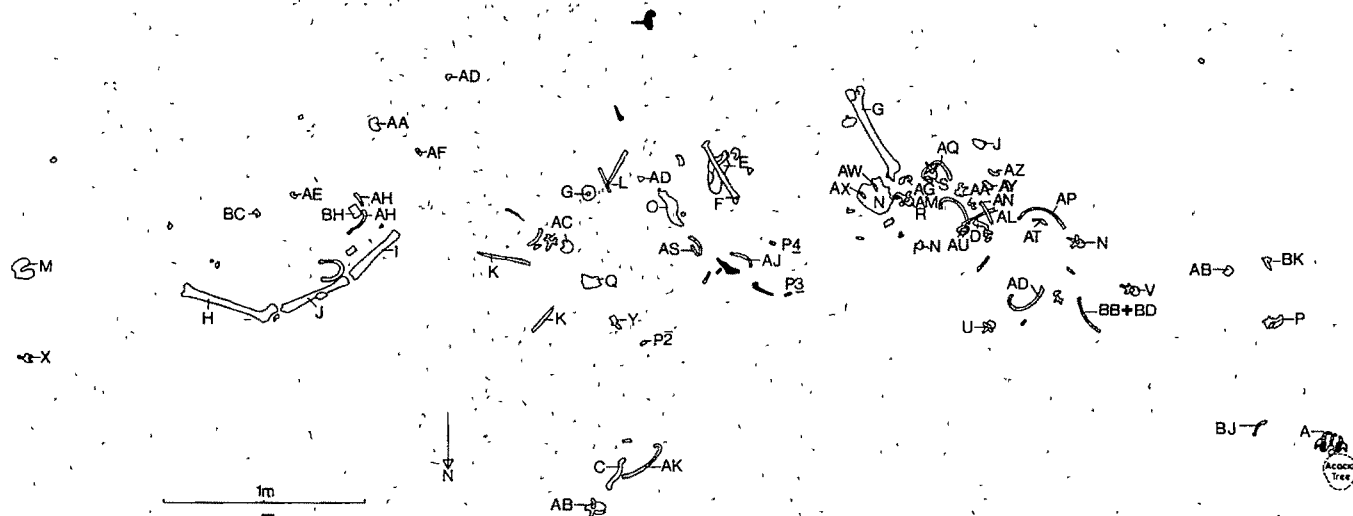


Fig. 3 Detail of site plan showing distribution of *H. erectus* bones. Major hominid parts are as lettered in Table 3. Non-hominid bones, mostly fish, are in solid.

Table 3 Skeletal parts of KNM-WT 15000

Cranium (A)	Right rib 4 (AI, BD, BE)
Mandible (B)	Right rib 5 fragment (BJ)
Cervical vertebra 7 (R)	Right rib 6 (AL)
Thoracic vertebra 1 (S)	Left rib 7 (AP)
Thoracic vertebra 2 (T)	Right rib 7 (AK)
Thoracic vertebra 3 (U)	Left rib 8 (AU)
Thoracic vertebra 6 or 7, spine and laminae (BI)	Right rib 8 (AM)
Thoracic vertebra 8 (V)	Left rib 9 (AO)
Thoracic vertebra 9 (W)	Right rib 9 (AS)
Thoracic vertebra 10 (X)	Right rib 10 (AJ)
Thoracic vertebra 11 (Y)	Left rib 11 (AN)
Lumbar vertebra 1, body and right lamina and inferior facet (AR, BA)	Left clavicle (C)
Lumbar vertebra 2 (AA, AV)	Right clavicle (D)
Lumbar vertebra 3, pedicle laminae and spine (Z)	Left scapula, spine and axillary border (BK, BL)
Lumbar vertebra 4 (AB)	Right scapula (E)
Lumbar vertebra 5 (AC)	Right humerus (F)
Sacral vertebra 1 (AD)	Left ilium (L, BF, BG)
Sacral vertebra 3, laminae and spine (BB)	Left ischium (Q)
Sacral vertebra, right half (BC)	Right ilium (O)
Sacral vertebra 5 (AE)	Right ischium (P)
Coccygeal vertebra 1 (AF)	Right pubic fragments (AW, AX)
Left rib 1 (AG)	Left femur (G)
Right rib 1 (AY, AZ)	Right femur (H, M)
Left rib 2 (AQ)	Left tibia (I)
Right rib 2 (AH)	Right tibia (J)
Left rib 3 (AT)	Left fibula (K, BH)
	Right fibula (L)

to the means for white North American adult males given by Schultz in 1937 (ref. 22). We have examined casts and originals of other early *H. erectus* postcranial bones and find that all of them are large. Stini²³ noted current secular trends of increase in body size in many human populations and wondered whether the trend was revealing a genetic potential left over from early hunter and gatherer ancestors. The new *H. erectus* data support this hypothesis.

The vertebrae show some interesting differences from those of modern humans. The spinous processes on all vertebrae recovered so far are relatively longer and less inclined inferiorly than their modern counterparts and the laminae are much less broad. This means that the marked imbrication of the laminae and spines of adjacent vertebrae (particularly the thoracic series) so typical of *H. sapiens* is much less marked. The vertebral foramen is smaller relative to vertebral body size in the lower cervical and thoracic series, unlike that of modern humans, but about the same in the lumbar series. The inferior articular facets of the fifth lumbar vertebra face almost directly anteriorly and are slightly concave, in contrast to the usual human condition in which the surfaces face laterally and are cylindrically convex²⁴.

Although we have yet to recover sufficient parts of the pubic bones to complete a full reconstruction, the innominates are very similar indeed to OH 28 and KNM-ER 3228 (refs 16, 25), but the strong iliac pillar had not yet developed. The sacrum is represented by S1 and S5, the spine of S3 and half of S4. Although we can reasonably estimate the sacral width, precise alignment with the pelvis must wait for the recovery of S2. There was a remarkable degree of iliac flare²⁶; this flare is concordant with the extremely long femoral necks, which are relatively as long as those of robust australopithecines²⁶. The biomechanical advantage of the abductor mechanism²⁶ was much enhanced relative to the condition in *H. sapiens*. The biomechanical neck length of 85 mm is well over 3 s.d. from the mean of a sample of *H. sapiens*²⁶. As well as having a long femoral neck, the neck-shaft angle is very small at 110°, being 5 s.d.s from the mean of the same *H. sapiens* population. The femoral head diameter at 44.0 mm is almost exactly on an *H. sapiens* mean²⁷ and scales with femoral length in the same way as *H. sapiens* and *Australopithecus boisei*²⁸.

Martin²⁹, following a stimulating discussion of human and great ape brain growth, suggested that early *H. erectus* could

have produced their adult brain sizes by modification of the rate and/or extent of fetal growth without any need for significant postnatal postponement of fetal growth patterns as found in humans. This is dependent, however, on *H. erectus* pelvis having allowed the birth of an infant as large as those of *H. sapiens*. Modern human populations show little, if any, sexual dimorphism in the absolute width of the sacrum³⁰, a major determinant of pelvic inlet size. The interacetabular distance in WT 15000 is very small and surely correlated both with the great iliac flare and long femoral necks. It seems likely to us that early *H. erectus* birth-canal diameters would have been significantly smaller than in *H. sapiens* and that passage of a modern-sized, full-term fetus would have been impossible. The continuation after birth of fetal growth rates, found only in

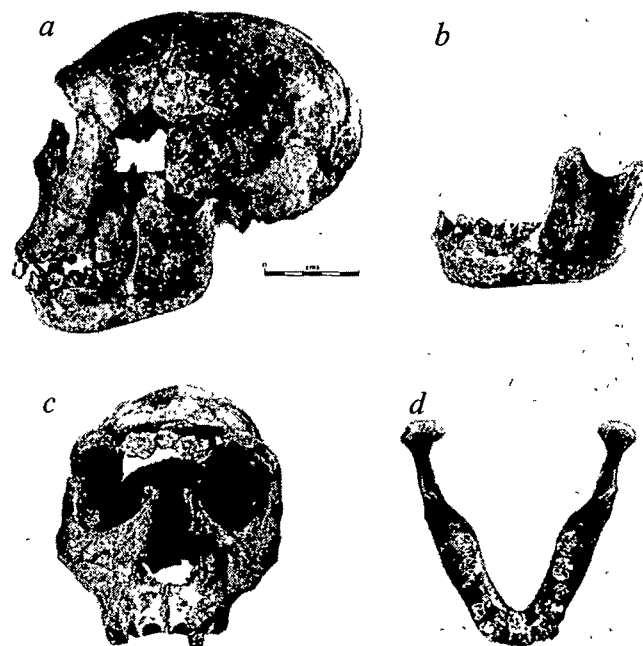


Fig. 4 KNM-WT 15000. a, Left lateral view of skull; b, left lateral view of mandible; c, frontal view of cranium; and d, occlusal view of mandible.

Table 4 Tooth measurements (mm)

	Right		Left	
	Mesiodistal	Buccolingual	Mesiodistal	Buccolingual
Mandible				
C	8.8	9.0	8.6	9.4
P ₃	9.0	10.1	8.5	10.1
P ₄	—	—	9.0	9.5
M ₁	11.9	11.1	12.2	10.9
M ₂	12.4	11.4	12.2	11.5
Maxilla				
I ²	7.9	8.5	7.9	8.3
P ³	8.3	11.5	—	—
P ⁴	8.2	11.5	—	—
M ¹	12.2	11.8	11.1	11.8
M ²	12.5	12.6	13.3	11.7

humans, must have been part of the developmental mechanism of *H. erectus*. Already then, by 1.6 Myr ago, the secondarily altricial condition (which leads to increased infant dependency) must have been present.

This skeleton has already shown that our views of early

H. erectus morphology must be modified. With the possibility of finding more parts when the excavation is continued and with further analysis, KNM-WT 15000 will provide a firmer foundation for the identification and understanding of unassociated postcranial bones and early hominid growth patterns. Except for those representing the latest stages of our evolution, it is also the first fossil hominoid, let alone hominid, in which brain and body size can be measured accurately on the same individual. These two crucial variables, on which so much speculation about human origins and behaviour has been based, can now be determined for at least one individual early hominid.

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LETTERS TO NATURE

Age of the Okote Tuff Complex at Koobi Fora, Kenya

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The Okote Tuff Complex crops out in the Koobi Fora region next to the north-eastern shores of Lake Turkana, northern Kenya. It occurs in the upper part of the ~450 m thick, essentially flat lying, Plio-Pleistocene sequence of fluvial, deltaic and lacustrine sediments comprising the Koobi Fora Formation¹⁻³. Many important archaeological sites and hominid fossils, including the *Homo erectus* finds discussed in the accompanying papers^{4,5}, are stratigraphically close to the level of the Okote Tuff Complex⁶ or its correlatives, so that its precise numerical age is of great interest. Previously published isotopic ages, mainly by the ⁴⁰Ar/³⁹Ar dating technique, exhibited a very wide scatter⁷. Here we report comprehensive K-Ar data showing that pumices within one of the tuffs of the Okote Tuff Complex have an age of 1.64 ± 0.03 Myr, which is in the earliest Pleistocene⁸.

The type locality for the Okote Tuff Complex is within area 131, adjacent to Karari Ridge, 20-km east of Lake Turkana (Fig. 1). The unit was formally named by Findlater² and discussed further by Cerling and Brown³. A stratigraphical section measured in this area is shown in Fig. 2, after ref. 5. The Okote Tuff Complex lies about mid-way between the KBS and Chari Tuffs which, in area 131, are separated by ~40 m of section consisting mainly of river channel sands together with some flood plain silts and clays. The Complex has yet to be defined and described in detail. In the field it consists of several sandy

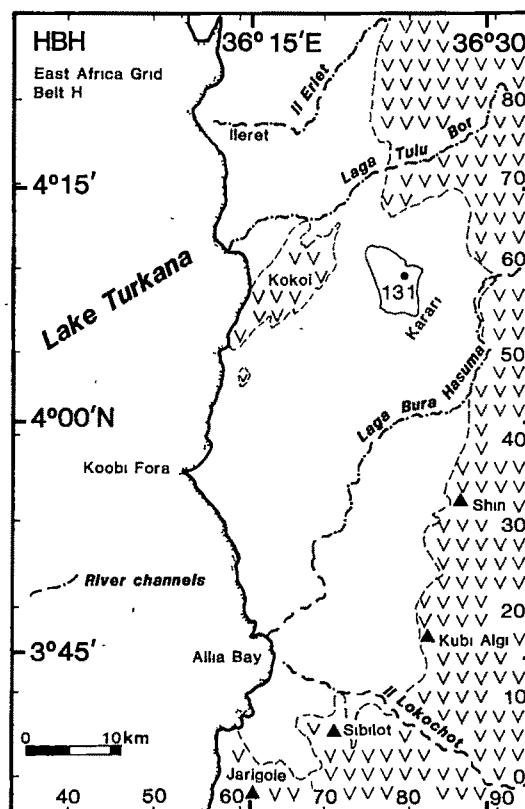


Fig. 1 Outcrop of the Koobi Fora Formation adjacent to the shores of Lake Turkana is indicated by blank area. Type locality for the Okote Tuff Complex is shown by filled circle within fossil collection area 131. Local basement, dominantly Neogene volcanics, is indicated by pattern.

Table 1 K-Ar age data for alkali feldspar phenocrysts from pumice clasts within a tuff bed of the Okote Tuff Complex, area 131, Koobi Fora, northern Kenya

Sample no.	K (wt%)	Radiogenic ^{40}Ar (10^{-11} mol g $^{-1}$)	100 Radiogenic ^{40}Ar Total ^{40}Ar	Calculated age ± 1 s.d. (Myr)	Average age ± 1 s.d. (Myr)
81-133	4.757, 4.737	1.395	43.3	1.694 $\pm$ 0.020	1.65 $\pm$ 0.05
		1.320	38.3	1.603 $\pm$ 0.020	
		1.361	33.2	1.652 $\pm$ 0.022	
81-134	4.745, 4.741	1.600	82.6	1.941 $\pm$ 0.018	—
83-265	4.728, 4.744	1.347	54.7	1.639 $\pm$ 0.018	1.65 $\pm$ 0.02
		1.368	47.3	1.665 $\pm$ 0.020	
83-272	4.698, 4.704	1.325	38.8	1.625 $\pm$ 0.020	1.64 $\pm$ 0.02
		1.351	38.6	1.657 $\pm$ 0.020	
83-326	4.712, 4.736	1.357	57.9	1.656 $\pm$ 0.020	1.64 $\pm$ 0.02
		1.338	47.7	1.632 $\pm$ 0.020	
83-328	4.715, 4.723	1.307	31.6	1.596 $\pm$ 0.021	1.62 $\pm$ 0.03
		1.338	25.6	1.634 $\pm$ 0.026	
83-329	4.682, 4.710	1.299	23.9	1.594 $\pm$ 0.025	1.60 $\pm$ 0.02
		1.313	42.4	1.611 $\pm$ 0.019	
83-330	4.694, 4.695	2.011	42.5	2.468 $\pm$ 0.028	—
83-331	4.751, 4.726	1.320	47.9	1.605 $\pm$ 0.019	1.64 $\pm$ 0.04
		1.391	36.9	1.692 $\pm$ 0.023	
		1.345	55.2	1.636 $\pm$ 0.018	
83-332	4.699, 4.702	1.390	60.7	1.704 $\pm$ 0.019	1.70 $\pm$ 0.02
		1.386	63.2	1.699 $\pm$ 0.019	

$\lambda_e + \lambda_c = 0.581 \times 10^{-10} \text{ yr}^{-1}$, $\lambda_\beta = 4.962 \times 10^{-10} \text{ yr}^{-1}$, $^{40}\text{K}/\text{K} = 1.167 \times 10^{-4} \text{ mol mol}^{-1}$. Mean age (excluding 81-134, 83-330) = 1.64 ± 0.03 Myr. Mass of sample used in Ar extraction in range 0.8 to 1.5 g. Locality: at coordinates 049-124 on 9 $\times$ 9-inch photographic print number 1750 taken in December 1970 by Hunting Surveys, London, where coordinates are millimetres from left edge and from the top edge of the print, respectively. Trimming of the pumices was done in the field to remove the outer centimetre or two of each clast to minimize the possibility of contamination of the samples by detrital feldspar. Some of the pumices were partly calcified. Separation of the feldspars from the pumice clasts was done, after crushing, on the >0.5 mm size fraction by water panning, followed by purification using heavy liquid and magnetic techniques. Most concentrates were washed ultrasonically for 3 min in 7% HF to help remove traces of glass adhering to the feldspar. Purity of >99% was attained. The crystals were quite unaltered and limpid, and exhibit very fine polysynthetic twinning. K and Ar were measured by flame photometry and isotope dilution, respectively. Ar extractions were made in a vacuum system by radiofrequency heating of the sample, contained in a Mo crucible, to temperatures >1,600 $^{\circ}\text{C}$ for 1 h to try to ensure full Ar recovery from the melt. After purification of the Ar, isotope analysis was carried out in a VG-Isotopes MM1200 gas-source mass spectrometer.

ryholitic tuff beds extending over ~ 16 m of section. Individual beds are generally 0.7–1.5 m thick. The tuffs were deposited from water in much the same manner as the enclosing detrital sediments.

In a small hill ~ 100 –150 m north of archaeological site FxJj20 at East African Grid HBH793592, at the type locality of the Okote Tuff Complex, rounded pumice clasts of up to ~ 20 cm in diameter are found in the upper part of a tuff bed 1.6-m thick, designated the Lower Okote Tuff⁵. A relatively large number of pumices was collected from this locality, as each contained some alkali feldspar phenocrysts, ideal for isotope dating by the conventional K-Ar method. Dating techniques used were similar to those described previously⁹.

Results on the alkali feldspar (anorthoclase) concentrates from 10 different pumice clasts are given in Table 1. Duplicate K analyses agree to better than 0.5% in most cases. Replicate Ar measurements show a spread averaging about 2%, with differences of >5% in two cases. For these two samples (81-133 and 83-331), the relatively large spread in their measured Ar contents may be because of minor but variable contamination by detrital feldspar, or because of incomplete extraction of the radiogenic Ar from some of the melts; with the available data it does not seem possible to distinguish between the alternative explanations.

The K contents of the 10 anorthoclase concentrates show a very limited spread, with a mean of 4.72 (± 0.02)%; this is good evidence that the pumice clasts were derived from a single volcanic eruption.

Eight of the alkali feldspars yielded K-Ar ages within the range 1.60–1.70 Myr, from which a mean age of 1.64 ± 0.03 Myr is calculated. The remaining two feldspars gave substantially

older apparent ages of 1.94 ± 0.02 and 2.47 ± 0.03 Myr (Table 1). These two results are clearly anomalous, probably to be explained by minor contamination of the mineral separates from old detrital feldspar incorporated within the pumice clasts.

The good agreement of the K-Ar results on eight of the feldspars, yielding a mean age of 1.64 ± 0.03 Myr, is accepted as strong evidence that this records the time of cooling of the feldspars immediately following the explosive volcanic eruption that produced the pumice clasts. Findlater¹⁰ argued that the time between explosive volcanism and deposition of the products is likely to be short in comparison with the time resolution obtainable by the K-Ar dating method. Brown and Feibel⁵ have shown, however, that the glass of the pumice clasts differs significantly in composition from that of the enclosing Lower Okote Tuff, implying that they are products of different eruptions. No tuffs in area 131 have been found that have glass of the same composition as that of the pumices, although a good match is obtained with tuff J4 in the Shungura Formation. As pumice clasts are likely to have been transported by floating¹¹, reworking and final incorporation into younger sediments is a reasonable explanation for their present location. Thus 1.64 ± 0.03 Myr must be the maximum age for deposition of the enclosing Lower Okote Tuff.

This limiting age for a tuff in the lower part of the Okote Tuff Complex is intermediate between that obtained for the Chari Tuff (1.39 ± 0.02 Myr; ref. 12) higher up the sequence, and the age of 1.88 ± 0.02 Myr (refs 9, 12, 13) found for the KBS Tuff ~ 16 m below, consistent with the stratigraphical position of the Okote Tuff Complex in the sequence.

The KBS Tuff and immediately adjacent sediments are normally magnetized, whereas the overlying sediments, including

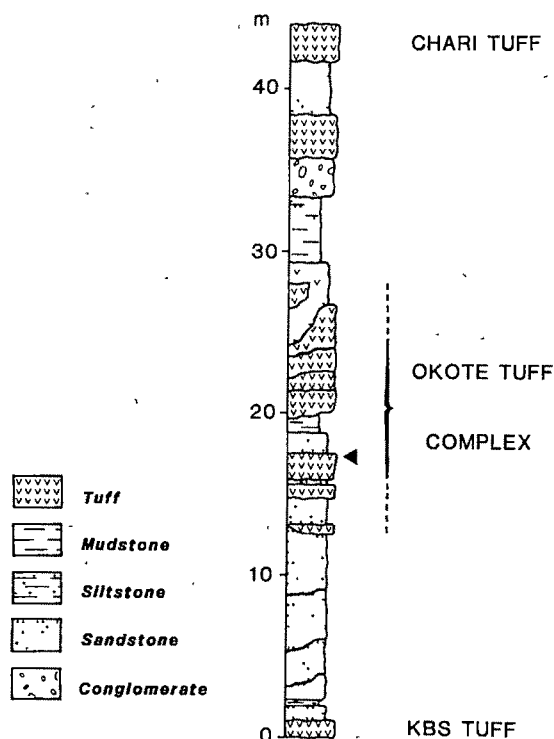


Fig. 2 Stratigraphical section through part of the Koobi Fora Formation in area 131, adjacent to Karari Ridge, after ref. 5. Pumice clasts dated in this study were collected from the Lower Okote Tuff, indicated by the symbol on the section ~17 m above the base.

the Okote Tuff Complex, are of reverse polarity^{14,15}. The palaeomagnetic and age data, therefore, are consistent with deposition of the sequence during the Matuyama reverse chron, the sediments at the KBS Tuff level having been laid down during the Olduvai normal subchron¹⁶.

In the Ileret area and adjacent to Koobi Fora spit (Fig. 1), tuffaceous beds also occur above the KBS Tuff in a stratigraphical position similar to that of the Okote Tuff Complex. Various called Ileret, Lower/Middle and Koobi Fora Tuff Complexes, these are thought to be stratigraphical correlatives of the Okote Tuff Complex^{2,3,5,11}. Cerling and Brown showed³, however, that glass separates from pumice and from tuff display considerable compositional variability, such that definite correlations between individual tuffs in the different areas was not possible. As the compositional variations observed lie on well defined trends, they suggested³ that the tuffs are all genetically related, and, therefore, probably correlatives on a broad time-scale. Studies by Brown and Feibel⁵ have subsequently yielded some correlations. The age derived here for pumices in one of the tuffs in the Okote Tuffs Complex can thus be regarded as providing an approximate guide to the age of the other tuff sequences referred to above.

The well-constrained maximum age for a tuff within the Okote Tuff Complex provides excellent time control for the abundant vertebrate faunas, including hominids, that occur in the upper part of the Koobi Fora Formation. Correlations indicate that the recent important *Homo erectus* find at Nariokotome on the west side of Lake Turkana also was from a stratigraphical level equivalent to that of the Okote Tuff Complex^{4,5}. Together with the dating results reported by McDougall¹² on feldspars from pumice clasts in other tuffs in the sequence, a detailed chronology for the Koobi Fora region is emerging. The successful geochemical correlation of tuffs between the sequences at Koobi Fora, the Lower Omo Valley just north of Lake Turkana (Shungura Formation) and the west side of Lake Turkana, strongly supports the idea that the sediments belong to a single major depositional system within the Turkana Basin^{3,5,17}. The chronology also can be applied throughout the region, with measurements on samples from different parts of the Turkana Basin contributing to the overall picture. The available data

suggest that accumulation of sediments in the Turkana Basin began ~4.1 Myr ago in the early Pliocene, and has continued until the present day, although quite lengthy hiatuses, representing periods up to or exceeding 0.5 Myr, are recognized in individual sequences^{12,17}.

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Stratigraphical notes on the Okote Tuff Complex at Koobi Fora, Kenya

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The Okote Tuff Complex of the Koobi Fora Formation in northern Kenya contains many archaeological sites¹ and many hominid specimens derive from levels nearby². The complex lies between the KBS Tuff and the Chari Tuff, both of which have been precisely dated^{3,4}, and recently McDougall (in the accompanying manuscript)⁵ has measured the age of pumice clasts derived from the complex. The pumices, however, differ in composition from the tuff in which they were deposited. The age is still useful, because a tuff of known stratigraphical position in the Shungura Formation is of the same composition. Here we discuss stratigraphical relations between the Okote Tuff Complex and other related strata at Koobi Fora, in the lower Omo Valley, and west of Lake Turkana based on compositional study of ash layers. This allows us to estimate an age of 1.60 ± 0.05 Myr for the *Homo erectus* skeleton recovered at Nariokotome in 1984 (see accompanying article⁶).

Several conditions have contributed to the difficulty of framing a general stratigraphy in this part of the section, or even to defining the sequence of tuffs which occur. The first is that in many localities only a single tuff or a pair of tuffs is exposed, and it is often difficult to determine the order of tuffs where exposures are poor because many of them occur discontinuously as channel fillings. Many of the tuffs in this interval have reasonably similar composition with respect to both major and trace elements; each of them is variable in composition so that where few analyses are available, samples which actually belong to a single group may be placed into different categories. Some of the tuffs are composed of shards of more than one composition⁷, and fractionation may have occurred either naturally (by differential weathering) or in the laboratory (by bias towards more or less magnetic fractions during electromagnetic separation). Finally,

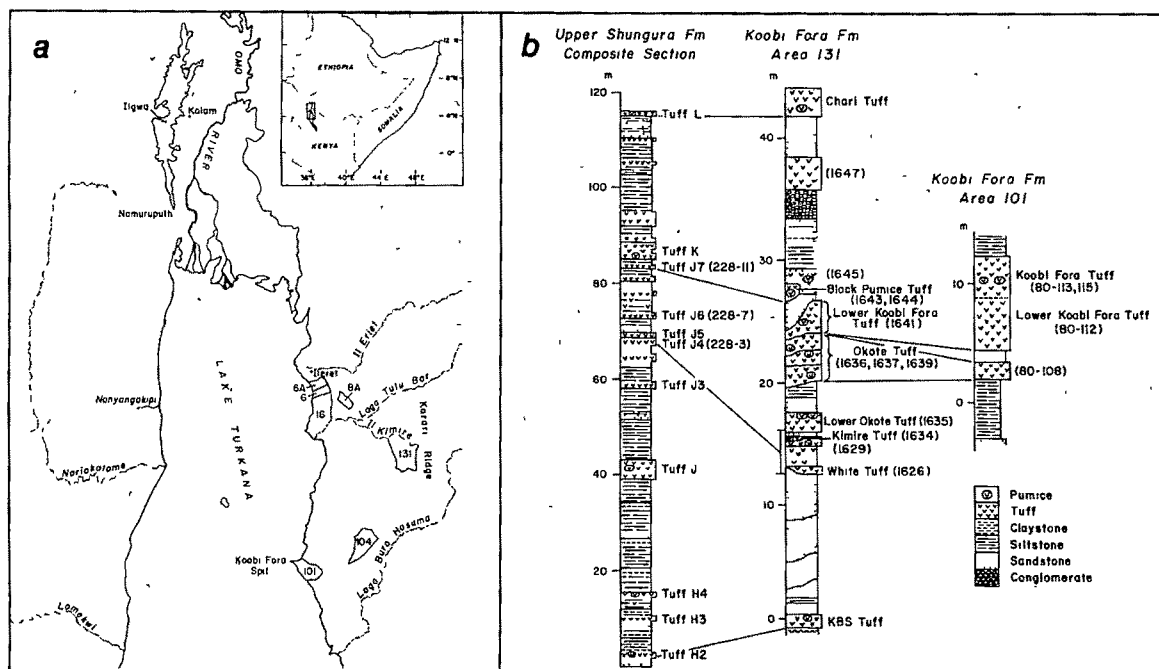


Fig. 1 a, Location map; b, Correlated stratigraphical sections through the Okote Tuff Complex in the lower Omo Valley and the Koobi Foraregion.

the sequence is obscured if analyses of pumice and fine-grained ash are considered together; only after separation of the pumice analysis from those of the tuffs does the situation become clearer. These problems can be overcome provided there are enough analyses to determine the variation in composition of a particular tuff, and provided the analyses are done on samples from more than one section so that sequences can be shown to be homotaxial. Stratigraphical statements made here are based on 143 individual analyses of glass from tuffs and from pumices contained within them which lie between the KBS and Chari Tuffs.

There are two key sections on which the present stratigraphical conclusions are based, one situated in area 131 on the Karari Ridge, the other situated in the lower Omo Valley (Fig. 1a, b). In a detailed section in area 131, there are 14 tuffs or tuffaceous layers beginning with the KBS Tuff and ending with the Chari Tuff. We view three layers near the middle of the section as products of a single eruption because they are compositionally identical. The section of the Shungura Formation is composite, but tuffs discussed here were collected from a single section at Ilgwa (see ref. 8); analyses are given in Table 1. A third section located near Koobi Fora spit is necessary to place the Koobi Fora Tuff (defined below) in this sequence.

On the basis of the stratigraphical order of tuffs in these sections, and the composition of the ash fractions, the correlations shown in Fig. 1b have been made. Appended names are explained below. We realize that this stratigraphy is incomplete—indeed we have analysed 10 other tuffs known to lie within this same interval, but which are less well constrained stratigraphically, and thus not reported here.

In previous reports concerned with the stratigraphy of this interval¹⁹⁻²⁰, the tuffaceous part of the section along the Karari Ridge was referred to as the Okote Tuff Complex (or as the Okote Tuff); at Ileret, the Lower/Middle Tuff Complex (or Middle Ileret Tuff); and near Koobi Fora spit, the Koobi Fora Tuff Complex. With the exception of the Okote Tuff Complex defined by Findlater¹³, these terms are informal. To clarify stratigraphical relations between the three areas at Koobi Fora we here name several tuffs which have formerly been treated as parts of one or another of the complexes. For each newly named tuff we designate a type locality and give a short description; representative analyses of each are given in Table 1.

A tuff in area 104 has been informally known as the White Tuff; it is also present in area 131 where it is the lowest recognized ash layer mapped as the Okote Tuff Complex. We define this tuff as the White Tuff, and designate its type locality as (1750/043, 103) (ref. 21) in area 131 of the Koobi Fora region where the tuff is reasonably well exposed, and where its relation to other tuffs in the Okote Tuff Complex can be seen. This tuff is ~40-cm thick, fine grained, and very pale grey to white. It is composed dominantly of colourless shards with tubular vesicles. Its very pale colour is diagnostic in the field and its composition is unique.

About 2 m higher in the section in area 131 there is a medium-grey tuff ~80-cm thick (K83-1629, Table 1) which we do not name. Filling a channel in the upper part of this tuff is another ash ~40-cm thick which we designate the type locality of the Kimire Tuff, named for the stream in which it is exposed. Photo coordinates of this locality are 1750/054, 131. (Photo coordinates refer to aerial photography HSL KEN 70 6 flown by Hunting Surveys Ltd. The notation is read as photo number/x, -y with the x distance in millimetres from the left hand margin of the image and the y distance in millimetres from the top of the image. A set of these photos exists in the Koobi Fora archives at the National Museums of Kenya in Nairobi.) Shards with spherical bubbles make up about half of the Kimire Tuff; the remainder are striated, bubble junction or bubble wall shards. This tuff cannot be distinguished chemically from the Lower Koobi Fora Tuff; it is separated here because the stratigraphical relations are clear. Isolated samples from other localities could be referred to either the Kimire Tuff or to the Lower Koobi Fora Tuff.

A thin sandstone lies between the Kimire Tuff and the next tuff which we designate the Lower Okote Tuff (Type locality 1750/057, 133). The pumices dated by McDougall⁵ derive from this horizon, but are chemically very distinct from the enclosing ash. The Lower Okote Tuff (1.6 m) is medium grey, fine to medium grained, and composed dominantly of shards with elongate vesicles with a smaller fraction of bubble junction shards. Although similar in composition to some other ashes in this same stratigraphical interval, the Lower Okote Tuff is reasonably well defined compositionally.

There are four pumiceous tuff beds 2 m above the Lower Okote Tuff, which comprise ~6 m of the section. The lower

Table 1 Representative analyses of tuffs and pumice from the Okote Tuff Complex

Sample no.	Location	Weight per cent		Elemental content (p.p.m.)								
		Fe ₂ O ₃	CaO	Ba	Mn	Nb	Rb	Sr	Ti	Y	Zn	Zr
Unnamed Tuff												
83-1647	131	4.89	0.55	143	2,154	148	113	4	2,642	84	191	993
Unnamed Tuff												
83-1645	131	4.89	0.29	48	1,510	139	116	3	2,319	102	211	1,086
Black Pumice Tuff												
81-615	Shin	2.38	1.12	719	1,077	76	83	129	2,692	63	97	708
228-11	Omo	2.46	1.12	740	989	80	77	151	2,731	60	95	661
83-1576	8A	2.91	1.51	751	960	70	77	187	3,792	53	96	701
83-1643	131	2.67	1.07	764	1,024	86	81	108	2,473	63	102	726
83-1644D	131	1.91	0.73	784	792	77	91	80	2,270	61	80	570
Koobi Fora Tuff												
228-7	Omo	5.64	0.27	40	2,112	241	143	3	2,682	127	290	1,576
80-113	101	5.35	0.30	10	1,862	252	120	6	2,176	130	306	1,658
80-115	101	5.39	0.19	<5	1,899	256	127	1	1,969	131	282	1,656
Lower Koobi Fora Tuff												
83-1641	131	4.83	0.30	35	1,463	145	122	11	2,208	106	224	1,164
80-112	101	5.27	0.49	35	1,600	163	107	20	2,413	104	226	1,198
80-163	103	5.00	0.37	35	1,485	142	108	12	2,064	99	215	1,096
Okote Tuff												
83-1636	131	4.75	0.18	10	1,759	242	145	1	1,902	121	265	1,542
83-1639	131	4.76	0.21	21	1,736	228	148	1	1,948	120	265	1,513
83-1637	131	4.69	0.23	12	1,546	214	149	6	1,835	121	255	1,516
80-108	101	4.70	0.46	8	1,610	214	118	4	2,494	114	241	1,396
Lower Okote Tuff												
83-1635	131	4.66	0.14	25	1,105	164	168	2	1,193	127	259	1,540
83-1575	8A	4.80	0.14	<5	1,073	167	157	1	1,097	128	257	1,580
Tuff J4												
228-3	Omo	3.31	0.43	262	816	141	84	4	1,321	102	196	1,176
ANU83-331	131	3.19	0.53	645	906	146	86	10	1,725	108	195	1,134
ANU83-328	131	2.73	0.34	193	721	153	95	3	1,462	111	207	1,022
ANU83-326	131	2.80	0.52	226	705	155	98	7	1,514	115	205	1,035
83-511	Turkana	2.96	0.37	218	710	157	93	1	1,111	116	245	1,047
Kimire Tuff												
83-1634	131	5.00	0.29	45	1,541	136	110	1	1,962	102	216	1,100
Unnamed Tuff												
83-1629	131	4.74	0.55	691	1,751	104	95	5	2,220	76	178	846
83-1629NM	131	4.74	0.52	670	1,635	104	95	4	2,201	77	193	850
White Tuff												
83-1626	131	1.84	0.17	26	247	112	159	2	502	87	117	474
80-169	104	1.86	0.18	<5	242	116	175	1	498	93	120	526
Tuff at Nariokotome below hominid KNM-WT-15000												
84-2957	Turkana	5.18	0.34	97	1,440	135	110	1	2,098	101	221	1,095

three of these are compositionally identical (Table 1), and as these comprise the greatest thickness of ash at this locality we designate these three beds the Okote Tuff. At the type locality (1750/061, 137) the Okote Tuff (4 m) is medium grey and consists of a mixture of shard types. Compositionally this tuff is well defined, and corresponds to a 1.4-m ash layer which lies ~1 m below the Lower Koobi Fora Tuff in area 101.

The fourth Tuff in the sequence described above correlates with the base of the thick tuff in area 101 which has been informally regarded as the type locality of the Koobi Fora Tuff⁸. Here we designate the tuffaceous section in area 101 as the type locality of both the Lower Koobi Fora Tuff and the Koobi Fora Tuff (1343/102, 173). There is no clear stratigraphical break between the lower and the upper part of the thick tuff in area 101, but the base is compositionally distinct from the upper part (see Table 1). In other parts of the Koobi Fora region, ashes of each composition are found as discrete layers. The lowest 4.1 m of section at the type locality is taken as the type of the Lower Koobi Fora Tuff; the upper 3.3 m of section at the same locality is taken as the type of the Koobi Fora Tuff. Both of these tuffs are composed dominantly of shards with tubular vesicles with a few blocky or bubble junction shards. The Koobi Fora Tuff

probably correlates with Tuff J-6 of the Shungura Formation, although there are minor differences in composition (Table 1). In area 131, the Koobi Fora Tuff has not been identified, probably because the Lower Koobi Fora Tuff is terminated there by an erosional surface with at least 3-m relief.

Encased within the sandy section overlying the Lower Koobi Fora Tuff in area 131 is a distinctive tuff composed of both grey and brown shards. There it occurs in a channel lined with ash, filled with black pumice clasts up to 25 cm in diameter, but also contains grey pumice clasts of similar size. We here designate this tuff as the Black Pumice Tuff. It is 0-1 m thick at its type locality (1750/060-139), pale brownish yellow in hand specimen, and composed dominantly of flat bubble junction shards. Compositionally this tuff, though variable, is unmistakable (Table 1); it correlates with Tuff J-7 of the Shungura Formation. (Two other separates have been analysed from the section in area 131 between the Black Pumice Tuff and Chari Tuff (K83-1645; K83-1647; Table 1, Fig. 2), but as they are known only from this locality, we do not name them here.)

Turning to the composition of the pumice clasts, we find that most can be accommodated in the analytical groups defined using ash samples only. One fact becomes immediately clear

from the analyses of pumice clasts—pumices are not confined to the tuff which they match compositionally, but may be found also in tuffs and other sediments at higher stratigraphical levels. For example, the Koobi Fora Tuff contains not only pumice corresponding to its own composition, but also pumices which compositionally match the Lower Koobi Fora Tuff and the Okote Tuff. This causes the problem of interpreting the ages on the pumices from the Lower Okote Tuff, because they do not correspond to the composition of that tuff. Clearly, the measured ages refer to the time of eruption of the pumice, and must antedate the time of deposition of tuff in which they are included. In a general sense, the effect of upward reworking of pumice clasts in a section is to make the entire section appear older than it actually is. In contrast to pumices, which maintain their integrity even during and after reworking, ash layers are not often redeposited as discrete layers. (There are, however, examples of redeposited ash layers in the region.) Hence, we argue that the age on pumice clasts must be applied stratigraphically to the first appearance of an ash in the section which corresponds to the composition of the dated pumice.

The pumices from the Lower Okote Tuff correspond in composition to Tuff J-4 in the Shungura Formation of southern Ethiopia, and also to a tuff exposed west of Lake Turkana, but do not correspond to that of any tuff yet analysed from the Koobi Fora region. We believe that the age reported by McDougall¹ may be applied to Tuff J-4 of the Shungura Formation. Using this age, we can estimate ages of tuffs lying between J-4 and Tuff L in the Shungura Formation by scaling on the basis of stratigraphical thickness. This in turn allows us to set limits on the age of the Lower Okote Tuff, knowing that it is 1.64 ± 0.03 Myr. Using various sections for scaling, the Black Pumice Tuff (Tuff J-7) is found to have an age between 1.52 and 1.57 Myr, hence the Lower Okote Tuff must lie between 1.64 and 1.52 Myr. If a further correlation is accepted (that between Tuff J-6 and the Koobi Fora Tuff), then the age of the Lower Okote Tuff is further constrained to lie between 1.60 and 1.64 Myr, which is virtually the same as the error in the reported age. In this case, the tuff in question is only slightly younger than the pumices included within it even though they differ in composition. The lowest tuff known to have been mapped within the Okote Complex is the White Tuff which can be assigned an age of ~ 1.7 Myr by stratigraphical scaling of the section in area 131.

Detailed correlations between the Karari Ridge and the Ileret area are still not completely clear. The Lower Koobi Fora Tuff and the Koobi Fora Tuff have been identified in areas 6, 6A and 8A in the southern part of the Ileret Area, and these lie above the Lower Ileret Tuff²⁰. We can set no useful lower boundary on the level of the Lower Ileret Tuff, although it is known to overlie the KBS Tuff. The Black Pumice Tuff has also been identified in area 8A. The prominent tuff exposed below the Chari Tuff along the northern part of the Ileret Ridge does not correlate with any of the tuffs reported here, nor with any other known tuff from the region.

In conclusion, the Okote Tuff Complex and the Koobi Fora Tuff Complex as a whole probably lie within the interval between 1.5 and 1.7 Myr. The stratigraphical interval between the KBS Tuff and the Okote Tuff Complex represents a period of no more than 200,000 yr. Several archaeological sites can now be placed into an ordered sequence along the Karari Ridge, and with further work others will undoubtedly be placed as well. The tuff which lies 50 cm below the *Homo erectus* skeleton announced in the accompanying article⁶ (KNM-WT-15000), on the west side of Lake Turkana correlates either with the Kimire Tuff or with the Lower Koobi Fora Tuff; until other tuffs lying lower in the section at the site are analysed it is not possible to decide which. But it is clear that the hominid specimen must lie between 1.55 and 1.65 Myr in age.

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A new form of energy dissipation by a moving object in He II

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Despite the superfluid^{1,2} character of ⁴He below the λ transition temperature, there are two distinct mechanisms by which an object moving through the liquid dissipates kinetic energy: (1) it can scatter^{3–5} the excitations (rotons, phonons, ³He isotopic impurities) that constitute the normal fluid component; and (2) for speeds in excess of the Landau critical velocity⁶ (v_L), it can create rotons⁷, apparently in pairs^{7,8}. The first process has been studied extensively, mostly by measurements of the zero-field mobilities of positive and negative ions^{3–5,9–11}. The second process, which is much rarer, has also been investigated in considerable detail^{12–14} by studies of the motion of negative ions in isotopically pure ⁴He at elevated pressures. Here, we report an attempt to extend the latter type of investigation to lower pressures. This yields unexpected results that can be accounted for satisfactorily only if we postulate the existence of a third dissipation mechanism.

All the experiments were carried out at temperatures low enough that the influence of process (1) could be ignored. At high pressures, P , the drift velocity, $\bar{v}$, of a moving ion is limited by roton pair creation. In weak electric fields, it is accurately described^{12,13} by

$$\bar{v} = v_L + AE^{1/3} \quad (1)$$

where the constant A can be expressed¹⁵ as

$$A = \left(\frac{5.36 e \pi^2 \hbar^3 v_L^2}{m_i k_{0i}^4 V_{k_0, k_0}^2 \mu_r} \right)^{1/3} \quad (2)$$

m_i is the ionic effective mass, k_0 and μ_r are the roton wave vector and effective mass and V_{k_0, k_0} is the matrix element, unknown *a priori*, that characterizes the pair-emission process. The experimental technique developed for the determination of $\bar{v}$ in weak electric fields has already been outlined^{16,17} and will be described in detail elsewhere¹⁸. Briefly, a group of ions is field-emitted into a large volume (~ 1.5 l) of isotopically purified liquid ⁴He at ~ 80 mK and their average velocity in traversing

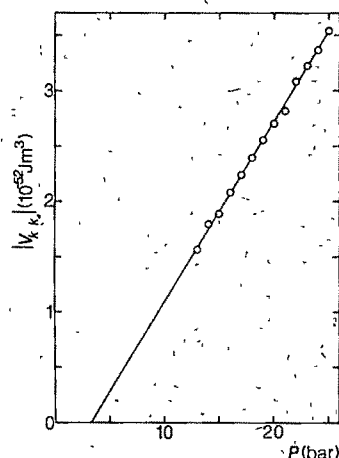


Fig. 1 The modulus of the matrix element $|V_{k_0, k_0}|$, for roton pair creation by negative ions in He II plotted as a function of pressure, P . The solid line has been fitted to the data by the method of least squares.

a region of weak uniform electric field is then measured by means of a time-of-flight technique. Comparison of the experimental $\bar{v}(E)$ data with equations (1) and (2) enables v_L and $|V_{k_0, k_0}|$ to be determined. The values of v_L are found¹⁶⁻¹⁸ to agree to within 1.5% with those calculated from the Landau theory⁶ on the basis of accepted values¹⁹ of the roton parameters. The experimental $|V_{k_0, k_0}|$, incorporating measured²⁰ values of m_+ , are plotted as a function of pressure in Fig. 1. It can be seen that $|V_{k_0, k_0}|$ decreases rapidly with falling P and that a linear extrapolation would imply that it became zero at ~ 3 bar. (Metamorphosis of the bare ions to charged vortex rings^{21,22} prevents the extension of this type of experiment much below 13 bar.)

Although there is no theoretical justification for a linear extrapolation *per se*, it seems reasonable to conclude from Fig. 1 that the probability of roton pair creation will become very small at low pressures. One possibility is that V_{k_0, k_0} may pass through zero and change sign at some characteristic pressure. Without an explicit physical model of the roton creation process, one can only guess at the underlying reasons for such behaviour. It seems probable that the most significant result of reducing P is the consequent expansion^{5,20} of the ion; it is conceivable that there may be some critical ionic radius at which the two rotors of a nascent pair will tend to interfere destructively, effectively reducing the probability of the emission event in question.

Regardless of the correctness of such speculations, it is of considerable interest to investigate the behaviour of bare ions moving through He II at low pressures: for low enough temperatures, in isotopically pure ^4He , process (1) can be disregarded; and the data of Fig. 1 strongly imply that process (2) will also be ineffective as a significant source of drag. What, then, will limit the speed of an ion moving under the constant force arising from an external electric field?

An experiment to investigate this question seems at first sight to be precluded by the rapidity with which bare ions undergo the transition to charged vortex rings at low pressures, thereby being lost from the propagating ion cloud whose drift velocity is to be measured. The transition can, however, be suppressed²³ by the application of electric fields $> 2 \text{ MV m}^{-1}$. Measurements in these conditions are not straightforward because of the need to maintain electric fields above this value throughout the whole transit of the ion from source to collector; but they are possible. We have performed a set of measurements of $\bar{v}(P)$ for $E = 2.6 \text{ MV m}^{-1}$ extending down to 1 bar (Fig. 2).

Although the data are subject to considerable scatter, it is immediately evident that the form of $\bar{v}(P)$ is very different from what might reasonably have been anticipated (Fig. 2). As P is reduced below 25 bar, $\bar{v}$ at first increases. This was as expected: first, because of the increase of v_L (dashed line) which causes $\bar{v}$ also to increase (so that, if $\bar{v} - v_L$ were to remain constant, the data would follow the dash-dotted curve); and, second,

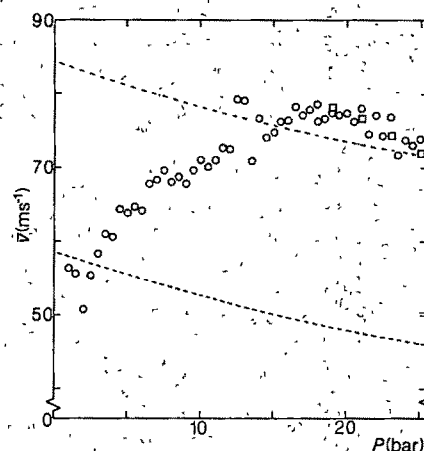


Fig. 2 The drift velocity $\bar{v}$ of negative ions in He II at 0.3 K under an electric field of 2.6 MV m^{-1} , plotted as a function of pressure, P . The dashed curve shows the pressure dependence of the Landau critical velocity for roton creation; the dash-dotted curve indicates the form of $\bar{v}(P)$ that would be expected if $(\bar{v} - v_L)$ remained constant as P was reduced from 25 bar. Squares represent data acquired through use of the electric induction technique described previously²² and circles represent measurements made with a modified (single grid) form of the same technique that has been devised to enable the electric field criterion in the text to be satisfied, and which will be described in detail elsewhere.

because of the decrease of $|V_{k_0, k_0}|$ which causes $\bar{v}$ to rise above the dash-dotted line as is indeed observed. This trend is not, however, continued to lower pressures. Rather, $\bar{v}(P)$ passes through a maximum at ~ 16 bar and then falls rapidly to reach a value near 1 bar that is actually below v_L . The latter observation clearly demonstrates that the drag force that limits $\bar{v}$ at the lower pressures cannot possibly arise from any form of roton emission. Therefore, it seems necessary to conclude that there exists some new dissipation mechanism as well as (1) and (2) mentioned above.

The average rate of energy dissipation by the ion may be written as

$$eE\bar{v} = 2\Delta R_2(\bar{v}) + \dot{\epsilon}_v \quad (3)$$

where the first and second terms on the right correspond, respectively, to dissipation through roton emission and through the unknown additional mechanism, Δ is the roton energy gap and $R_2(\bar{v})$ is the roton pair creation rate. Knowing $\bar{v}$, v_L and the matrix element for pair production (Fig. 1) we can calculate^{7,15} the rate of energy loss through roton creation; by subtraction of this quantity from our measured values of $eE\bar{v}$ we then obtain $\dot{\epsilon}_v$. Application of this procedure to the $\bar{v}(P)$ data of Fig. 2 yields the $\dot{\epsilon}_v$ values shown in Fig. 3. The error bars show the effect of a $\pm 5\%$ uncertainty in the matrix element and the random error in the data is indicated by their scatter. It is evident that the additional dissipation mechanism at first intensifies

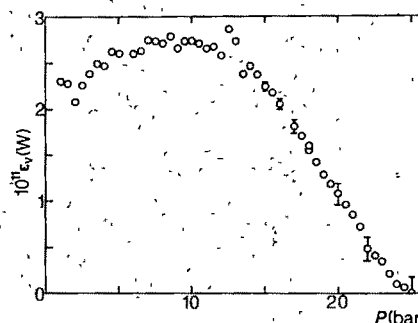


Fig. 3 The rate of energy loss, $\dot{\epsilon}_v$, caused by the new dissipation mechanism for a negative ion moving in an electric field of 2.6 MV m^{-1} . The points, calculated as described in the text from the data in Fig. 2, are plotted as a function of pressure, P . Bars indicate the effect of a $\pm 5\%$ change in the value used for $|V_{k_0, k_0}|$.

slightly with increasing pressure, passes through a maximum at ~ 8 bar and then falls quite steeply, approaching zero near 25 bar.

We propose that a probable candidate for this unknown source of drag lies in the continuous production of microscopic quantized vortex rings. That is, rather than undergoing the well-known transition to a charged quantized vortex ring, the ion creates a succession of elemental rings to which it does not bind. Such a phenomenon is anticipated²⁴ on the basis of Schwarz and Jang's symmetrical creation model^{25,26} of the transition; it could conceivably also occur for the alternative vortex loop model²⁷ if the elemental loop were to become unstable in very high electric fields. We infer that the process can happen at all pressures but that it does so much more weakly at the higher values of P , mainly because of the opposing pressure dependences of v_L and the critical velocity v_c for vortex nucleation. The existence of such a phenomenon would also provide a plausible explanation for the observed small departures¹⁴ of $\bar{v}(E)$ from the behaviour predicted by the roton pair emission theory for large E at 25 bar.

Two specific points arise concerning the relationship of our present results to the large body of work on the interaction of ions with vortices in He II. First, it is well established that, for non-zero temperatures, there is a finite probability of an ion escaping from the vortex on which it is trapped, as the result of a thermal fluctuation²⁸⁻³⁰. In some conditions, therefore, the ion can proceed through the liquid in a series of distinct epochs such that it exists for part of the time as a bare ion and for the rest of the time forms part of a charged vortex ring³¹⁻³⁶. Initially, the ion accelerates under the influence of the applied electric field, and provided that its velocity $v \geq v_c$, the transition to a charged vortex ring may occur. The ring grows and slows and, eventually, particularly for strong electric fields, the ion may escape from the ring through a thermal fluctuation^{31,35,36}; it then starts to accelerate again, and so on. (In those cases where the electric field is inadequate to maintain the stability of the ring against process (1), but still sufficient to cause $v \geq v_c$, the ring shrinks, accelerates and disappears, again liberating the trapped ion^{32,34}; the process then repeats, as before.) The mechanism envisaged here is distinctly different in character, however, in that charged vortex rings as such are not created because trapping of the ion on the nascent ring does not occur: the 'moving object' therefore maintains its continuity of existence as a bare ion. Second, the notion that a moving ion might be able to create a sequence of uncharged microscopic vortex rings was proposed several years ago by Huang and Olinto³⁷. Their suggestion related to a theoretical explanation for the Careri mobility steps which, unfortunately for the theory, were subsequently shown to have been, if not an artefact, then some classical phenomenon quite unconnected with the quantized vorticity of the superfluid under consideration. But, although incorrect in its original context, their proposal can provide a basis for the interpretation of the present experiments.

We may interpret Fig. 3 in the following way. The rate of energy loss to vortex rings can be written

$$\dot{E}_v = \nu E_{\text{ring}} \quad (4)$$

where ν is their nucleation rate and E_{ring} is their average energy. The dissipation rate through this process becomes very small at large P because, for any given velocity, rotons are then more strongly emitted: this is partly a result of the decrease of v_L , partly because of the increase in $|V_{k_0, k_0}|$ and partly as a result of the increase of v_c . The dissipation rate also exhibits a slight decrease at low P , mainly because the ion is travelling more slowly, which is attributable to the decrease of v_c with falling P . The data in this range seem to favour the Muirhead *et al.* theory²⁷ of vortex nucleation slightly over that of Bowley²⁶, for which the predicted pressure dependence of v_c is weaker.

The situation is, however, complicated, first, because of the changes to be anticipated in E_{ring} owing to the pressure dependence of the ionic radius and, second, because of our ignorance of the distribution function of ionic velocities in these conditions. The vortex nucleation rate itself cannot, of course, be evaluated without a knowledge of E_{ring} ; but, if we make the plausible

assumption that the radius of the ring will be ~ 2 nm, slightly larger than the radius of the ion, so that $E_{\text{ring}} \approx 5 \times 10^{-21}$ J, we find from equation (4) that $\nu \approx 4 \times 10^8 \text{ s}^{-1}$ at zero pressure. The latter value seems physically reasonable.

In conclusion, it seems that a third form of drag exists on a moving object in He II, and we suggest that it corresponds to the production of a stream of quantized vortex rings.

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Image distortion near galaxies: dwarfs or lensing?

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Gravitational lensing is a useful tool for studying the mass distribution in galaxies. The average gravitational lens distortion of background galaxy images by foreground galaxies is an independent non-kinematical measurement of galaxy mass distribution M/r (ref. 1), where M is the mass of the galaxy and r its radius. Using an indirect calculation involving estimated luminosity functions and absolute magnitudes, Philipps² recently claimed that our measurement of field galaxy mass distribution by galaxy-galaxy gravitational lensing was contaminated by large numbers of foreground dwarf galaxies. I present here a direct measurement of this effect: the cross-correlation between these samples clearly reveals clustering of foreground dwarf galaxies, with an amplitude slightly less than that given by our original simulation¹. Thus, this effect does not change our conclusions regarding the average galaxy mass distribution.

Galaxy mass can be expressed as an equivalent orbit velocity at some radius. The upper limit we obtained for the equivalent

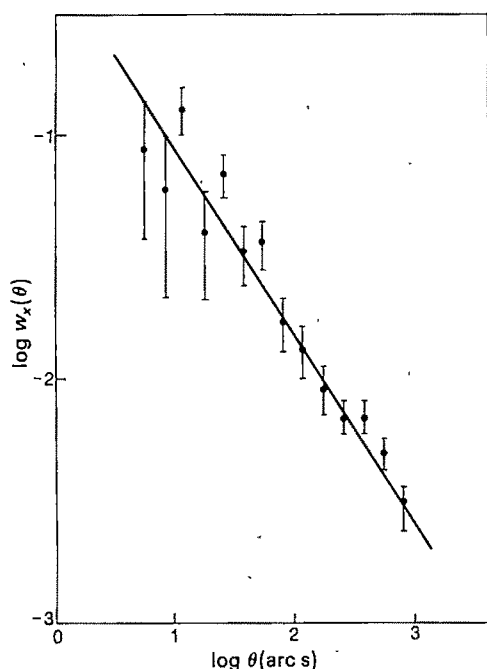


Fig. 1 Dwarf galaxies clustering about bright galaxies. The two-point cross-correlation function is plotted for the foreground galaxies and background galaxies used in the determination of galaxy mass distribution by gravitational light deflection¹. Note that contamination of the background galaxy data set by sub-luminous clustered foreground galaxies is small ($\sim 10\%$) at 10 arc s angular separation between the bright and faint galaxies. Even-odd bin round-off effects at small separation angle have not been corrected. The amplitude of this correlation function is 12 times less than that predicted by Phillipps², and is in reasonable agreement with our simulation.

circular velocity, while small compared with some heavy halo models, is consistent with dynamically derived estimates for a sample of galaxies of all types¹. For example, for a mean mass cut-off radius of $65 h^{-1} \text{ kpc}$, our 3σ upper limit for the equivalent circular velocity $\langle v_c \rangle = [(GM)/r]^{1/2} = 190 \text{ km s}^{-1}$, [h is the value of the Hubble parameter in units of $100 \text{ km s}^{-1} \text{ Mpc}^{-1}$]. The search for a statistical gravitational-lens image distortion of background galaxy images about the positions of foreground galaxies is complicated by several low-level systematics. We obtained upper limits to many systematics by substituting either random positions or stars for the foreground or background galaxies. Remaining contaminating effects, which are largest at small angular separations, must be modelled, simulated or, even better, measured directly.

Our foreground sample of galaxies has magnitudes in the range 19–21.5 J mag. Our background sample covers 22.5–23.5 J mag. A fraction (originally estimated¹ at 15%) of our 'background' galaxies are actually sub-luminous galaxies physically clustered with our foreground galaxies. This effect is one of several known systematics of which we were aware and which are corrected¹. However, a direct measurement is always better than a calculation.

The average magnitude of galaxies in our foreground sample is 20.75 J mag and that of our background sample is 23.06 J mag. Thus, any physical companions of the foreground galaxies, appearing as background galaxies, have absolute magnitudes about 2 J mag fainter than the average foreground galaxy in our sample.

I can test the hypothesis of significant dwarf galaxy contamination of our background sample by direct internal analysis of our data, without reference to any assumed luminosity function. Phillipps claims that large numbers of dwarf galaxies clustering about our foreground galaxies dilute our true background sample by a factor of 4–1.5 over the range 4–40 arc s separation angle, giving a cross-correlation function³ $w_x(\theta) = 9\theta^{-0.8}$, where

$w_x(\theta)$ is the excess probability, over random, of finding a faint galaxy near a bright galaxy, separated by an angle θ . This prediction by Phillipps is a necessary condition for his conclusion; that is, if $w_x(\theta)$ for our observations falls significantly below $9\theta^{-0.8}$, for whatever reason, this hypothesis fails.

Taking the same foreground and background galaxy data sets used in our galaxy mass determination¹, and forming the two-point cross-correlation function, I obtain the correlation shown in Fig. 1. There were 1.3×10^7 galaxy pairs in the cross-correlation, with average magnitudes of 21.04 and 23.06 J mag in the foreground and background samples, respectively. The best-fit line has the same slope (-0.77) found in our galaxy autocorrelation functions. I obtain (3σ):

$$w_x(\theta) = (0.7 \pm 0.1)\theta^{-0.77} \quad (1)$$

This is a factor of 12 below Phillipps' estimate, and is close to our original¹ estimate, based on a Monte-Carlo simulation using a Schechter luminosity function. In the range of θ that dominates our gravitational lens distortion measurement ($0.7 < \log \theta < 1.5$), our measured $w_x(\theta)$ averages $< 10\%$. Our original plots of image distortion were corrected for 15%. Thus, our equivalent circular velocities were overcorrected by 5%, and the effect of our new calibration of this effect is slightly to enhance our null result, the conclusions of our paper being unchanged. The formal discrepancy between Phillipps' estimate and our observations is 38 s.d. Thus, intrinsically faint galaxies do not significantly contaminate our background sample. Below I briefly discuss the apparent origin of Phillipps' anomalously large amplitude.

To estimate this contamination effect indirectly, we require the luminosity function in some standard system, and then we must transfer J magnitudes of average and dwarf galaxies onto that system. Using $B = J + 0.23(B - V)$, $B - V = 0.8(J - F)$ and the mean $B - V$ colours of average and dwarf galaxies at 21 J mag (1, 0.3), I obtain $B_{fg} = 21.2$ mag and $B_{fg} = 23.0$ mag for the mean apparent B magnitudes of these two galaxy type samples in our foreground sample. The colour-dependent K -corrections at $J = 21$ mag ($z = 0.2$) will be different for the dwarf and average galaxy samples. I estimate the difference in the colour terms as $\Delta K = 0.8$ mag. Since $B - M_B = 25 - 5 \log h + 5 \log r + K$, I obtain $M_{Bd} - M_B = 2.4$ mag for the difference between the dwarf and average galaxy absolute magnitudes in our foreground sample. If $\langle M_B \rangle = -20$, then $\langle M_{Bd} \rangle = -17.6$ mag.

Historically, published values for the galaxy luminosity function, without regard for the depth of the sample, have varied widely. Phillipps chose the value $0.05 h^3 \text{ Mpc}^{-3} \text{ mag}^{-1}$ from a range of observed values⁴. Recent deeper and more statistically homogeneous galaxy samples^{5–7} have given differential luminosity functions at $M_B = -17.6$ mag ranging from 0.006 to $0.015 h^3 \text{ Mpc}^{-3} \text{ mag}^{-1}$. Using $0.01 h^3 \text{ Mpc}^{-3} \text{ mag}^{-1}$, Phillipps' result would be within a factor of two of our measurement, well within the errors of his estimate. Felten⁸ argues for even lower values for higher redshift samples of field galaxies. Another theoretical estimate for this effect, which would predict negligible contamination for our sample, can be found in ref. 9, Table 4.

In this instance, due to uncertainties in published galaxy luminosity functions, a direct measurement of the two-point cross-correlation function of our foreground and background galaxy samples is more reliable than any calculation based on assumed luminosity functions and K -corrections. Given some foreground galaxy redshifts, cross-correlations in these faint galaxy samples may eventually yield new data on the faint end of the galaxy luminosity function.

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Intermittent baroclinic instability and fluctuations in geophysical circulations

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The presence of blocking highs in atmospheric circulation, bringing periods of stable weather alternating with more variable weather patterns, is an example of two distinct modes of behaviour occurring under similar conditions in a natural system. The occurrence of two or more different flow regimes under a given external forcing is a consequence of nonlinear dynamics that allows the system to equilibrate in different modes. Each mode can be stable over a range of conditions and yet there may be sudden transitions between modes. We have now discovered in a laboratory experiment using rotating fluids a case of at least two modes of behaviour under identical conditions. In one mode, a weak continuous forcing drives a mean circulation that is steady but on which are superimposed waves whose amplitudes vacillate under the influence of viscous dissipation. Under suitable conditions there is an alternative mode in which the mean flow fluctuates and unstable waves occur only in short bursts at periodic intervals separated by relatively long periods of stable flow with no waves. The existence of multiple equilibrium states is of interest in the climatology of all geophysical and astrophysical fluid bodies. Our discovery of a mode involving intermittent bursts of instability and long-period fluctuations of mean flow (resulting purely from internal dynamics rather than from variations in external forcing) may have implications for the variability of oceanic circulation or solar radiation on timescales of a few years to centuries.

The condition of solid-body rotation (or zero motion relative to a rotating reference frame) can never be attained by a fluid containing gradients of temperature or chemical composition¹. This is because diffusion of density across the curved surfaces of constant density drives a meridional (Eddington-Sweet) circulation in planes parallel to the axis of rotation^{2,3}. Conservation of angular momentum of the circulating fluid in turn leads to an even larger azimuthal flow with an associated vertical shear and finite inclination of surfaces of constant density relative to surfaces of constant pressure. This azimuthal flow can, under suitable conditions, be unstable to perturbations that convert gravitational potential energy into kinetic energy of waves or of irregular eddy motions superimposed on the mean circulation⁴; this mechanism for conversion of energy is termed baroclinic instability.

In our experiments, a cylindrical container of radius $R = 30$ cm and with a planar base perpendicular to gravity, rotates

with angular velocity Ω about an axis through its centre and parallel to gravity. A layer of water 10 cm deep is allowed to approach solid-body rotation before a dilute sugar solution ($0.5\text{--}5 \times 10^{-3} \text{ g g}^{-1}$) is slowly released onto the base from a small tube against the wall. Once a lower layer has accumulated to a depth of 10 cm, the pipe is removed and the top of the container sealed to prevent surface stress and evaporative cooling. Vertical motions of the density interface are detected by conductivity probes on the axis of rotation and at a radius of 20 cm. Each probe consists of a platinum wire electrode suspended vertically through the interface⁵. A small amount of salt is mixed with the upper layer before filling to produce a conductivity contrast between layers and the circuits are completed through a flat plate electrode on the wall of the container.

The vertical position z of the density interface on the axis of rotation is a convenient measure of the azimuthal circulation. In fact, by considering conservation of momentum and volume in each layer, it can be shown that variations of z at the centre of the cylinder are caused primarily by variations of a horizontally averaged velocity difference between layers (the radial pressure gradient resulting from the sloping interface being everywhere in approximate balance with Coriolis forces). Waves are not detected at the axis when the flow is unstable because they have a node at the centre and a maximum amplitude somewhere near the wall. On the other hand, conservation of volume implies that mean circulations have little influence on interface height 20 cm from the axis, where the second probe is located, so that the latter detects only waves and eddies.

After filling and a period during which relative motions induced by the filling procedure decay, diffusion of sugar across the thin interface begins to have a significant effect. Baroclinic instability of the resulting azimuthal flow depends on the internal Froude number $F = 4\Omega^2 R^2 / g(\Delta\rho/\rho)h$ (h is the depth of both layers, g the gravitational acceleration and $\Delta\rho/\rho$ the fractional density difference), the influence of friction expressed in terms of the Ekman number $E = \nu/2\Omega h^2$ (ν is the kinematic viscosity) and the Rossby number $Ro = \Delta U/2\Omega R$ (ΔU is a characteristic velocity difference^{4,5}). Waves are predicted to grow when $F > 0(10)$ and $E^3 Ro^{-1} < 0(1)$. In the present system, the velocity difference ΔU is a function of solute diffusivity, interface thickness and curvature of density surfaces (or $\Omega^2 R/g$). The above criterion, using a velocity scale measured from floating markers, predicts instability for all experiments in which the outer probe detected the growth of waves. As predicted, no waves appeared in experiments for which $F < 10$. Although the Eddington-Sweet circulation driven by solute diffusion has been detected previously in laboratory experiments⁶, its instability has not.

Other phenomena detected in our experiments are wave amplitude vacillation and the influence of instability on the mean flow. In Fig. 1 the waves persist with a mean amplitude that remains nearly constant over long times, apart from obvious amplitude vacillations that are a common feature of finite amplitude baroclinic waves^{5,7-10}. At the same time the mean azimuthal circulation is practically invariant, implying a balance between spatially averaged dissipation and forcing over all timescales.

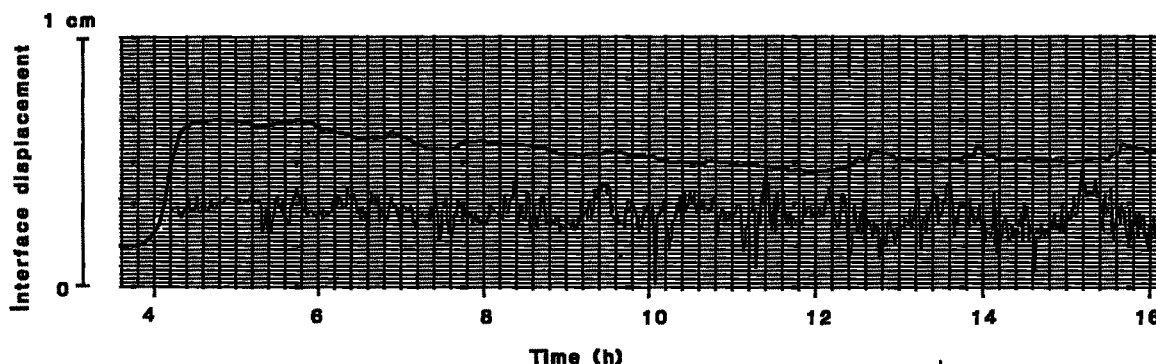


Fig. 1 Vertical displacements of the density interface as functions of time after filling as detected by conductivity probes at the axis of rotation (heavy line) and at a radius of 20 cm (lower trace) for an experiment in which $\Omega^2 R/g = 4 \times 10^{-2}$, $E = 8 \times 10^{-3}$, $Ro = 0.03$ and $F = 76$; $\Omega = 1.2 \text{ rad s}^{-1}$, giving a rotation period of 5.2 s. Baroclinic waves are continuously present and the mean flow is essentially steady.

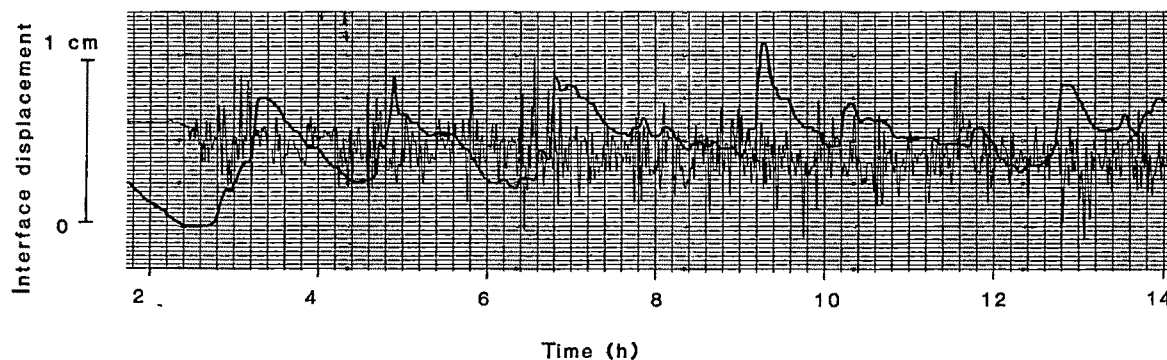


Fig. 2 Vertical displacements of the density interface in an experiment similar to that of Fig. 1 but with $F \approx 150$. Rotation period, 5.2 s. Baroclinic waves are continuously present (light trace) but induce fluctuations in the mean flow (heavy line).

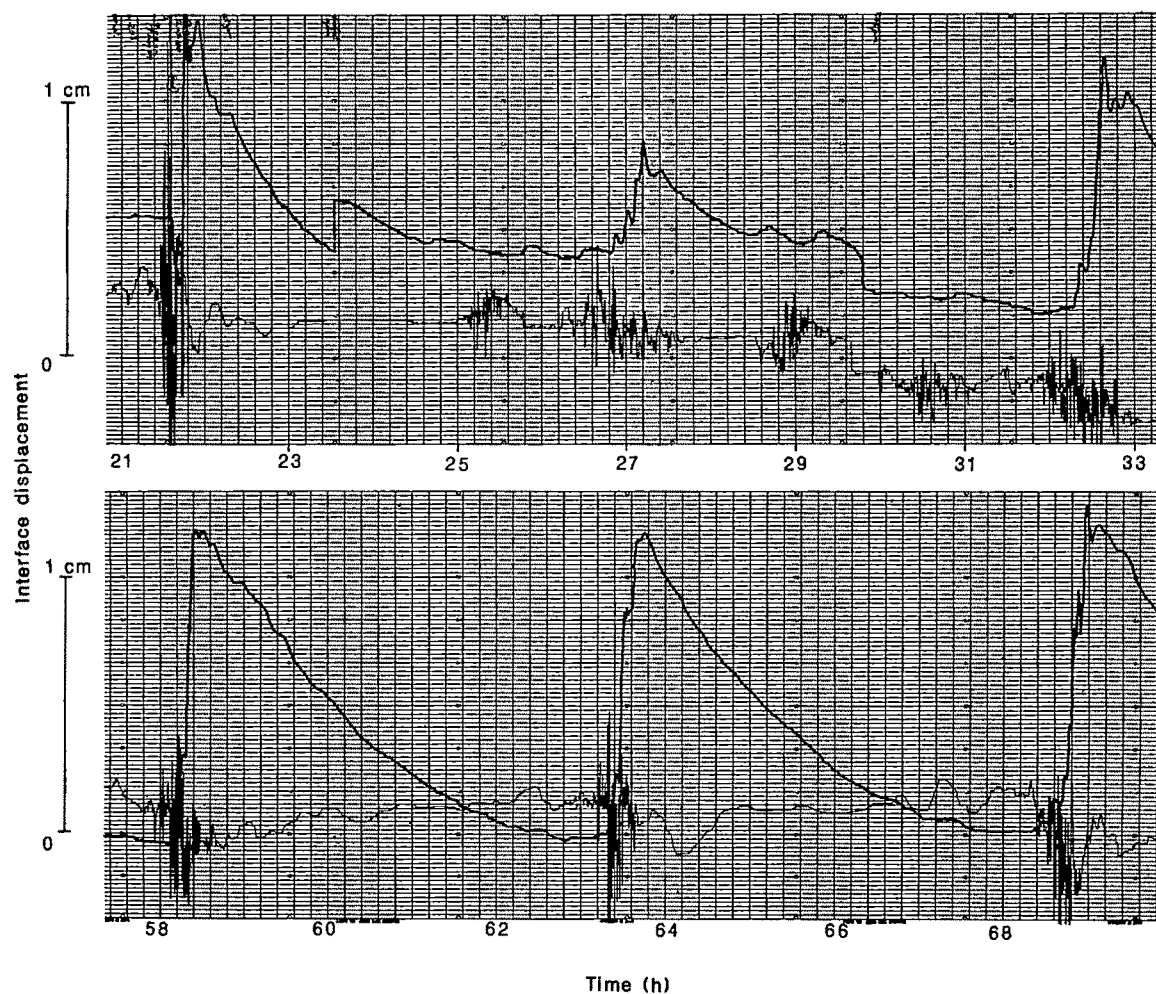


Fig. 3 Displacements of the density interface during two separate periods for the experiment of Fig. 1, showing the effect of an 8% increase in Ω at 21.6 h after filling. Baroclinic waves appear only intermittently (light trace) and the mean circulation (heavy line) eventually settles into a period oscillation. In this experiment, the periodic oscillations persist for 80 h until diffusion has so altered the density distribution that waves dies out.

When the experiment is repeated with the density difference halved, waves are again continuously present (Fig. 2). However, they show larger fluctuations of amplitude, and there are marked fluctuations in the mean azimuthal flow. In this case it seems that the forcing and dissipation are not in balance even over timescales up to hundreds of rotation periods.

The interaction of unstable waves with the mean flow can give rise to two flow regimes in a given experiment. A transition can be achieved by inducing a small instantaneous increase in

the angular velocity of the container. Figure 3 shows the result of increasing Ω from 1.2 rad s^{-1} to 1.3 rad s^{-1} in the experiment of Fig. 1. Immediately after Ω is changed, the lower layer is spun out towards the wall by a bottom Ekman layer and the interface falls at the axis of rotation. Within a couple of rotation periods (10–20 s), waves attain very large amplitude and can be observed when looking horizontally through the container. Such observations, as well as closed-circuit video monitoring of markers floating on the free surface, reveal that closed eddy

circulations form as the waves reach maximum amplitude. Waves and eddies then redistribute mass and angular momentum across the radius, reducing vertical shear and lifting the interface at the axis to well above its initial height. Without a sufficient interfacial shear to sustain them, the waves and eddies are rapidly dissipated by Ekman suction over a time of 10^2 rotation periods. However, isobaric surfaces must still be curved and diffusion again proceeds to slowly build up a mean azimuthal flow and vertical shear. The upper layer motion is clockwise relative to the container, which rotates in the anticlockwise sense. Hence the flow increases the curvature of density surfaces and further stimulates forcing due to the Eddington-Sweet mechanism.

After a period during which the system settles down, the flow in the experiment of Fig. 3 is stable for long periods of time (~ 5 h or 4,000 rotation periods), but is punctuated at regular intervals by relatively short bursts (lasting ~ 400 rotation periods) of unstable waves and eddies. Instability seems to occur whenever the vertical shear exceeds a magnitude beyond which release of potential energy by azimuthal waves overcomes dissipation of perturbations (E^2/Ro becomes small enough). It then causes a rapid readjustment of the mean circulation (taking as little as 150 rotation periods in the case shown). The cycle is repeated for as long as there is a sufficiently strong forcing: both baroclinic waves and long-period oscillations of the mean flow die out only when the interface becomes very thick and concentration gradients very small. The shortest period for oscillations of the mean flow found in any experiment is 1,100 rotation periods, although a wider range of conditions needs to be investigated.

The different rotation rate is not in itself significant in those experiments perturbed by a small change in Ω , as experiments that begin with the higher value of Ω but with other parameters equal also behave in the manner shown on Figs 1 and 3, with waves persisting in a continuous fashion until a change in Ω is impressed on the system. Thus, two flow regimes can occur under a given set of parameter values and in a given experiment. The regime realized must be dependent on the initial conditions or history of the flow.

Both the multiplicity of flow regimes and the possibility of energy release in short periodic bursts may be of relevance to circulation and heat transport through radiative zones in stellar interiors, where Eddington-Sweet circulations caused by thermal gradients have been predicted to be significant^{11,12}, but where only the stable and continuously unstable flow regimes have been considered. The possibility of a regime involving large fluctuations in mean circulation on timescales of the order of 1,000–5,000 rotation periods suggests the possibility of periodic variations of solar energy output on scales of tens to hundreds of years (using a rotation period for the Sun of 25 days). In oceanic circulation, the effects of other energy sources such as surface wind stress and meridional temperature gradients are expected to dominate over those of Eddington-Sweet circulations. However, on the scale of ocean basins, these forces too drive unstable baroclinic flows⁴ in which the interaction of nonlinear instability and mean flow might lead to multiple flow regimes or circulations fluctuating on long timescales. Again, if forcing and dissipation are such that mean flow fluctuations do occur, the oscillation period must scale with the rotation period of the system and our experiments indicate timescales of 3–15 yr. Data on the intensity of western boundary currents are insufficient to test for such fluctuations.

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Garnet phase of MgSiO_3 , filling the pyroxene-ilmenite gap at very high temperature

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Melting experiments on MgSiO_3 , the major constituent of the Earth's mantle and planetary interiors, have now been conducted at a static pressure of 20 GPa. These experiments reveal the presence of a new garnet-like non-cubic phase of MgSiO_3 , which melts congruently at 2,250 °C. This new silicate phase and its stability have significant implications for mantle mineralogy, chemical fractionation by partial melting and also for the occurrence of aluminium-deficient cubic garnet (majorite) in shocked meteorites. These high-pressure experiments at temperatures $>2,000$ °C have been made possible by the development of a large-volume high-pressure device at Nagoya University^{1–4}.

The high-pressure and high-temperature experiments were carried out using an MA8-type high-pressure apparatus^{1–4} and were made under a fixed ram load, which generated a pressure of 20 ± 2 GPa. To generate a very high temperature constant enough to melt silicates in a high-pressure cell, a composite of tungsten carbide and diamond was used for the heating element. The run temperature was measured by a W/W26%Re thermocouple, the hot junction being in contact with the graphite sample capsule. A powder of synthetic orthoenstatite was used for the starting material. Heating was limited to a few minutes to avoid contamination of the run product with the pressure medium, which was made of sintered MgO. Thin sections for observation by microscope and electron microprobe were prepared from the samples (~ 1 mm³), which were recovered after isobaric quenching following pressure release.

The run product at 1,760 °C, which show a granular texture indicating that recrystallization occurred in the solid state, can be identified by powder X-ray diffraction as the ilmenite phase of MgSiO_3 . The run product at 2,000 °C shows a more finely grained texture and consist of crystals with very low birefringence. The preservation of the starting composition can be

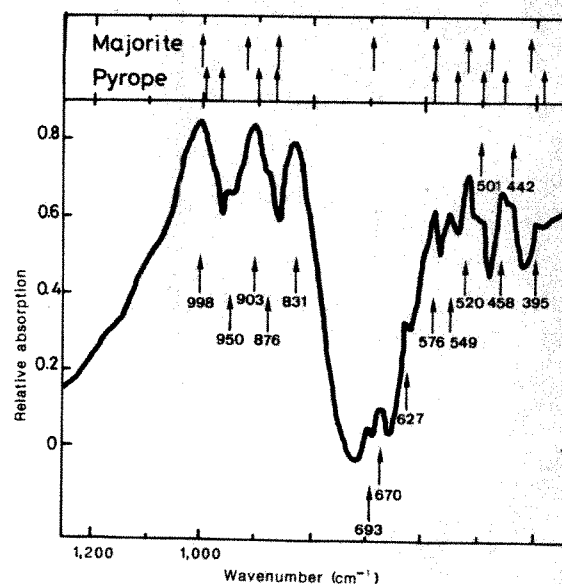


Fig. 1 Infrared absorption spectrum of non-cubic garnet of MgSiO_3 (1.8-mg sample in 190-mg KBr). The arrows at the top indicate the peak positions for majorite observed by Jeanloz¹¹ and for pyrope garnet observed by Moore¹². The present data show a similarity to those of two cubic garnets except for the presence of mode splitting.

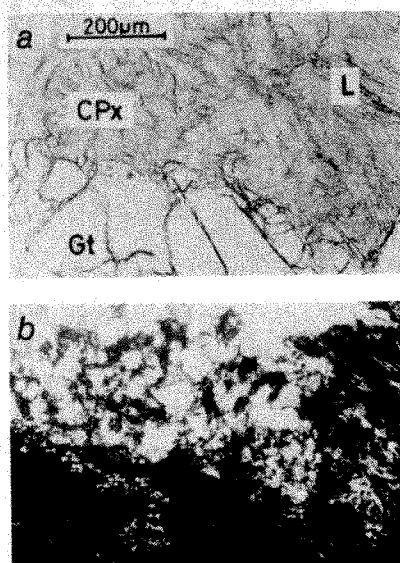


Fig. 2 Photomicrographs of the run product at 2,250 °C. *a*, Open nicol; *b*, crossed nicols. L, liquid phase; CPx, clinopyroxene; Gt, non-cubic garnet.

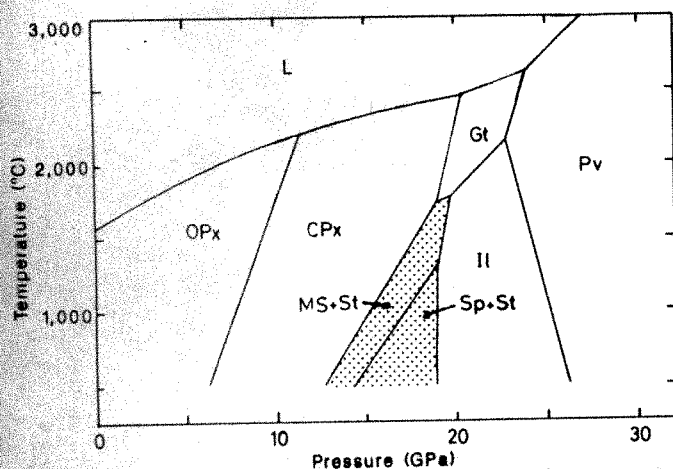


Fig. 3 Phase diagram for the composition of MgSiO_3 . The stippled region is the pyroxene-ilmenite gap in polymorphic transition in MgSiO_3 . The melting curve is drawn after ref. 17 below 5 GPa, and is extrapolated to higher pressures. OPx-CPx boundary is after Kanzaki (unpublished data). Il-Pv boundary is after ref. 20. Other phase boundaries are arbitrarily drawn to fit the experimental observations¹⁸⁻²⁰ and phase rule.

confirmed by electron microprobe analysis, which indicates $\text{MgO} = 50.5 \pm 1.0$; $\text{SiO}_2 = 49.5 \pm 1.0$; $\text{Al}_2\text{O}_3 < 0.1$ in mol%. The pattern of powder X-ray diffraction generally fits that of pyrope garnet (ASTM 15-742), although the peaks shift towards a low diffraction angle and some of them show splitting. A garnet phase with a low crystallographic symmetry has been reported in some silicates and germanates, for example, high-pressure polymorphs of MnSiO_3 (ref. 5) CdGeO_3 (ref. 6) and CaGeO_3 (ref. 7) with tetragonal symmetry. The observed peak splitting indicates that the present garnet-like non-cubic phase (referred to hereafter as non-cubic garnet) is probably isomorphic to tetragonal MnSiO_3 , and different from majorite, an aluminium-deficient cubic garnet found in shocked meteorites^{8,9}. The powder X-ray diffraction data for the non-cubic garnet of MgSiO_3 are tentatively classified as having tetragonal symmetry (Table 1). The mode splitting is so small that detailed assignment may involve some ambiguity; the uncertainty in lattice parameters is of the order of $\pm 0.01 \times 10^{-10}$ m. Sawamoto and Kozaki¹⁰ report lattice parameters of $a = 11.472 \times 10^{-10}$ m, and $c = 11.398 \times 10^{-10}$ m, and suggest that the distortion of cubic garnet of MgSiO_3 to non-cubic phase is associated with a density

Table 1 Powder X-ray diffraction data for MgSiO_3 garnet

Intensity	d_{obs} (10^{-10} m)	d_{calc} (10^{-10} m)	hkl
		4.685	211
10	4.668	4.668	112
		3.069	321
10	3.061	3.064	312
5	3.045	3.056	213
65	2.870	2.873	400
35	2.846	2.852	004
100	2.566	2.569	420
		2.565	402
50	2.553	2.554	204
		2.446	332
60	2.441	2.442	323
30	2.343	2.343	422
20	2.334	2.334	224
30	2.252	2.253	431
		2.248	413
30	2.243	2.243	314
		2.253	510
		2.253	501
		2.238	105
		2.097	521
10	2.096	2.096	512
5	2.082	2.085	215
		2.031	440
		2.024	404
20	1.8631	1.8637	611
10	1.8499	1.8510	116
10	1.8178	1.8169	620
		1.8510	602
		1.8048	206
20	1.6539	1.6545	444
20	1.5921	1.5935	640
25	1.5912	1.5899	604
20	1.5849	1.5853	406
35	1.5359	1.5347	642
50	1.5334	1.5323	624
35	1.5301	1.5282	426

$$a = 11.491 \times 10^{-10} \text{ m}, c = 11.406 \times 10^{-10} \text{ m}.$$

increase of about 1%. They are now trying to synthesize large single crystal samples for more accurate characterization of the crystallography.

Infrared spectral measurements of this phase and the result are plotted in Fig. 1 with the peak positions reported by Jeanloz¹¹ for natural majorite, $(\text{Mg}_{0.79}, \text{Fe}_{0.21})\text{SiO}_3$, and those reported by Moore *et al.*¹² for natural pyrope garnet. The data indicate mode splitting in this low-symmetry garnet.

Figure 2 shows a photomicrograph of the run product at 2,250 °C, in which clinopyroxene and non-cubic garnet are identified by powder X-ray diffraction. Figure 2b consists of non-cubic garnet crystals and Fig. 2a of clinopyroxene crystals. Both minerals show granular texture, while the right-hand side consists of a clinopyroxene and non-cubic garnet assemblage with a fine fibrous texture, which indicates quenching from the melt at high pressure. The electron microprobe analysis shows the same composition ($\text{MgO} = 50.4 \pm 1.0$, $\text{SiO}_2 = 49.0 \pm 1.0$, $\text{Al}_2\text{O}_3 = 0.6 \pm 0.3$ mol%) in these three phases (clinopyroxene, non-cubic garnet and melt). A little contamination during heating by H_2O and dopants from the pressure medium material has been found in the present system by Kato and Kumazawa⁴. Contamination of alumina is crucial in synthesizing the Al_2O_3 -free garnet. However, its content is so small that the phase stability may not seriously be affected. A small amount of H_2O should have little effect on the melting mode in this system. The above observations indicate that the experimental conditions (20 GPa, 2,250 °C) are very close to the triple point of clinopyroxene, non-cubic garnet and isochemical melt. Above this triple point, MgSiO_3 melts congruently at higher pressures.

The presence of alumina-deficient iron-magnesium garnet (majorite) in shock-metamorphosed meteorites has indicated

the possibility of its formation in pure MgSiO_3 garnet at high pressures and high temperatures. Ringwood and Major¹³ have reported the synthesis of garnet with a composition of $\text{Fe}/\text{Fe} + \text{Mg} = 0.25$ and $\text{Al}_2\text{O}_3 = 1.3$ mol % at 1,000 °C and above 25 GPa. Akaogi and Akimoto¹⁴ found that, in the enstatite (MgSiO_3)-pyrope ($\text{Mg}_3\text{Al}_2\text{Si}_2\text{O}_{12}$) system below 1,200 °C, at least 5 mol % Al_2O_3 is required to stabilize garnet. We have confirmed the expected formation of alumina-free garnet of MgSiO_3 at much higher temperatures than the previous studies. The synthesized garnet is not cubic but of lower symmetry. In some meteorites, cubic majorite garnet occurs in association with glass and high-pressure phases of orthosilicate (spinel and modified spinel)¹⁵, suggesting that crystallization involves melting at high pressures. This phase assemblage suggests that pressure and temperature conditions of majorite formation are close to those of the present experiment. The only difference between majorite garnet and the present non-cubic phase is the composition of $\text{Fe}/\text{Mg} + \text{Fe}$. Therefore, we anticipate the future finding of non-cubic aluminium-free garnet instead of cubic majorite in shocked enstatite chondrites (E) and bronzite chondrites (H), which have low $\text{Fe}/\text{Mg} + \text{Fe}$ in silicate phases. The detailed relationship between the cubic and non-cubic garnets is an important problem to be studied in order to clarify quantitatively the conditions prevailing in majorite-bearing meteorites.

The pressure-induced phase transitions in magnesium silicates (Mg_2SiO_4 and MgSiO_3) have been extensively studied because of their importance for investigating the nature of the Earth's mantle¹⁶. The transition in MgSiO_3 at ~1,000 °C is: orthopyroxene (OPx) → clinopyroxene (CPx) → modified spinel (MS) + stishovite (St) → spinel (Sp) + St → ilmenite (Il) → perovskite (Pv), and it possesses a gap between CPx and Il in polymorphic transition. In contrast, the transition in Mg_2SiO_4 shows a simple mode: two steps of polymorphic transition followed by a disproportionation reaction: olivine → MS → Sp → Pv + St. The disproportionation reaction of CPx involving stishovite, followed by the recombination reaction to Il, is poorly understood. The stability field of the new non-cubic garnet borders those of CPx and Il, all in the polymorphic relation, and the non-cubic garnet fills the gap between CPx and Il in the polymorphic transition of MgSiO_3 at high temperature. Therefore, MgSiO_3 has five polymorphic phases, OPx → CPx → Gt* → Il → Pv, in continuous sequence with increasing pressure (Gt* denotes non-cubic garnet). The presumed phase relation for MgSiO_3 composition is shown in Fig. 3, which summarizes the latest experimental data¹⁷⁻²⁰. The two phase assemblages involving stishovite (stippled area in Fig. 1) at the lower temperatures may be interpreted as a result of the abnormally high density and low entropy of stishovite.

We note several important implications of the present result for the constitution of the Earth and planetary interiors. The congruent melting of MgSiO_3 persists continuously from pyroxene to perovskite through non-cubic garnet, so that the pyroxene-stoichiometric phase remains as a major solid phase in the partial melting process throughout the mantle, regardless of the alumina and other minor elemental concentrations of the candidate material. Because the density of garnet is expected to be larger than the silicate melt throughout the upper mantle²¹, it may sink, thereby constituting the bottom of the upper mantle. The fractionation of garnet could have an important role in the formation of the layered structure in the mantle.

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Neogene history of the calcite compensation depth and lysocline in the South Pacific Ocean

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The depth-time pattern of calcite accumulation recorded at Deep Sea Drilling Project (DSDP) sites beneath the oligotrophic subtropical gyre in the south-east Pacific Ocean allows us to define here both the calcite compensation depth (CCD) and the lysocline. The Neogene CCD history is one of shoaling before 15 or 20 Myr and subsequent deepening to the present level of 4,100 m. A broad zone of increased calcite dissolution, 600 m thick, formed during early to middle Miocene time and has remained. This zone, the lysocline, denotes an important change in ocean chemistry and may reflect either an increase in the volume of more corrosive deep water beginning about 18 Myr and reaching steady state by 14 Myr, the time of rapid ice build-up in Antarctica, or an increase in calcite rain rate caused by an increase in surface-water productivity.

Early workers in marine geology realized that throughout most of the deep ocean the calcium carbonate microfossil tests generated in surface waters dissolved before they could be incorporated into the sediments¹. Experiments in the Pacific by Peterson² and Berger^{3,4} showed that calcite dissolution did not increase linearly with depth but that little dissolution occurred above about 3,500 m and rapid dissolution occurred at greater depths. This depth zone of rapidly increasing dissolution rate is the lysocline^{4,5} and that depth at which the dissolution rate of calcite matches the supply rate, and below which there is no net CaCO_3 accumulation, is the CCD^{6,7}.

Studies of piston cores⁸ and surface sediments⁹ from the South Pacific have shown the present CCD to be at ~4,100 m depth, and the top of the lysocline near 3,500 m. Heath¹⁰ studied calcium carbonate deposition in the equatorial Pacific and inferred changes in the CCD through Cenozoic time. Berger¹¹ first incorporated seafloor subsidence history into Pacific CCD studies, using an empirical age-depth curve similar to that compiled by Sclater *et al.*¹² Results of this study of equatorial Pacific sediments gave the first reasonable estimate of the equatorial Pacific CCD history. An important event in this history was the rapid deepening of the CCD from ~4,000 to 4,800 m at 35-40 Myr. Van Andel and co-workers used empirical subsidence curves and DSDP data to determine the CCD histories of the equatorial and non-equatorial Pacific¹³⁻¹⁵. Their results refined those of Berger for the equatorial Pacific and

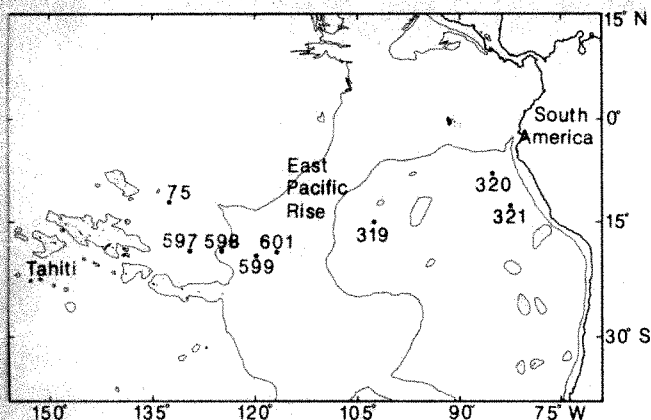


Fig. 1 Index map of the South Pacific showing sites used to construct the subsidence, CCD, and lysocline history of the region.

showed that CCD has been ~600 m shallower (4,000–4,200 m) since the early Oligocene in areas 10° or more north and south of the Equator.

We have determined the history of CaCO_3 deposition in the South Pacific based on data from the site chapters in the Leg 8 (ref. 16), Leg 34 (ref. 17) and Leg 92 (ref. 18) DSDP Initial Report volumes, converting the other data to the Leg 92 biostratigraphy. For each of the eight sites analysed (Fig. 1) we determined intervals of CaCO_3 deposition at rates typical of oligotrophic oceans, intervals over which there was an order-of-magnitude reduction in the mass accumulation rate of CaCO_3 -rich sediments, and the times at which CaCO_3 accumulation stopped¹⁹. The latter is a functional definition of the sedimentary CCD⁹. We presume that the large decrease in rate records the influence of the lysocline. The times of these changes are plotted along the subsidence curves¹⁹ and produce the first reconstruction of separate histories of the CCD and lysocline in the Pacific (Fig. 2).

The eight sites chosen for this study provide a generalized history of the lysocline and the CCD. Site 601 has always been above the lysocline. Sites 598 and 599 are now below the lysocline and above the CCD; Sites 319 and 597 have passed through both the lysocline and the CCD. The two oldest sites, 75 and 321, passed through the CCD abruptly with no evidence of reduced calcite accumulation rates before reaching the CCD. Site 320 was spot-cored; the youngest calcareous sediments recovered there are 15 Myr old. Nonetheless, the reasonably good agreement between the CCD from Site 597 and the maximum age of the transition through the CCD at 320 suggests that these sites passed through the CCD at about the same time.

The CCD in the South Pacific was at ~4,200 m in late Oligocene time, shoaled ~500 to a depth of 3,700 m in the middle-early Miocene, and has deepened since then to the present depth of ~4,100 m (Fig. 2). This history is similar to that determined by van Andel *et al.*¹⁵ for the subtropical and temperate Pacific.

The absence of a zone of decreased preservation²⁰ or reduced CaCO_3 accumulation above the CCD at Sites 75 and 321 suggests that the lysocline was not well developed before 20–17 Myr. It then developed and shoaled to 600 m above the CCD by 13.5 Myr. This 600-m dissolution zone (shaded in Fig. 2) has been present ever since. Linear sedimentation rates for CaCO_3 -rich sediment above the lysocline at Sites 597, 598, 599 and 601 and above the CCD at Sites 75 and 321 are all ~3–10 m Myr^{-1} . Site 319 had higher rates: 11 m Myr^{-1} just above the CCD and 35 m Myr^{-1} for older, shallower sediments¹⁹.

All investigations of the Cenozoic history of the CCD have shown a shoaling of the CCD from the middle Oligocene to middle or late Miocene time, followed by a younger deepening, broadly similar to the information shown in Fig. 2^{15,21–23}. A rise in the CCD must reflect some combination of (1) increased CO_2 content, hence corrosivity, of deep waters, reflecting either

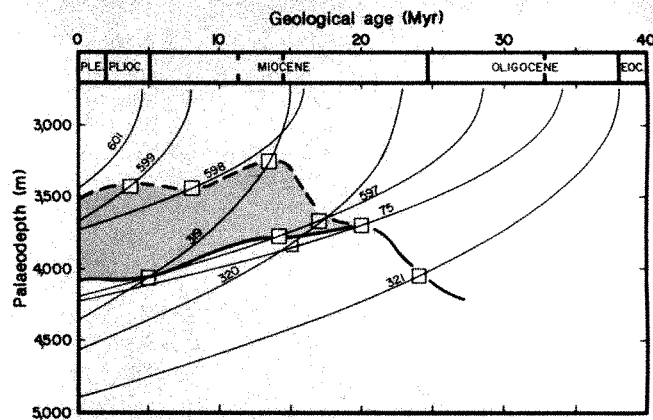


Fig. 2 CaCO_3 accumulation record plotted on age–depth curves for each site. Squares are 1 Myr $\times$ 100 m and provide an indication of accuracy. The shaded region indicates the depth zone of the lysocline. Solid line, CCD; dashed line, lysocline.

stagnation or enhanced input of organic carbon, and (2) reduced input of biogenic CaCO_3 to the deep ocean. The ultimate causes of changes in these parameters have been related to: the rate of formation of oceanic bottom water; the nature of shelf/basin depositional fractionation which depends largely on sea level; and sea-surface biological productivity of calcium carbonate and organic carbon. Oxygen isotope data suggest that the middle Cenozoic is characterized by constant or perhaps even declining ice volume^{24,25}, hence only minor changes in the production rate of bottom water may have occurred then. Sea level rose gradually from the mid-Oligocene to the mid-Miocene²⁶, consistent with a shoaling CCD, but as the sudden, large mid-Oligocene sea-level fall does not affect the CCD, it seems inappropriate to invoke the ensuing gradual rise of sea level. We therefore agree with the view^{15,22,23} that, during times of constant ice volume, sea-surface biological productivity exerts the dominant control of the CCD. The problem remains complex, however, because factors such as total carbonate supply to the sea floor¹⁵, the amount of non-calcareous, siliceous productivity²⁷, and how or why productivity changes during times of relatively uniform oceanic and atmospheric circulation, are not well understood on a global scale.

Van Andel *et al.*¹⁵ have suggested that the lysocline may be related to the volume of corrosive deep water. If this is the case, our data suggest an increase in this volume which, within the accuracy of our data, occurred during the same time span as the inferred ice build-up on Antarctica, 16.0–12.4 Myr (oxygen-isotope stratigraphy from ref. 28; foraminifer zonation and timescale from ref. 29). We note, however, that the present lysocline does not correspond to any water mass boundaries in the Pacific. More recently, Broecker and Peng³⁰ have suggested that the thickness of the zone of rapid CaCO_3 dissolution between the lysocline and the CCD varies directly with the downward flux of calcite. If so, the early Miocene separation of the lysocline and CCD may be related to increased surface-water productivity. There exists ancillary evidence supporting increased equatorial productivity of both calcareous¹⁵ and siliceous²⁷ organisms then.

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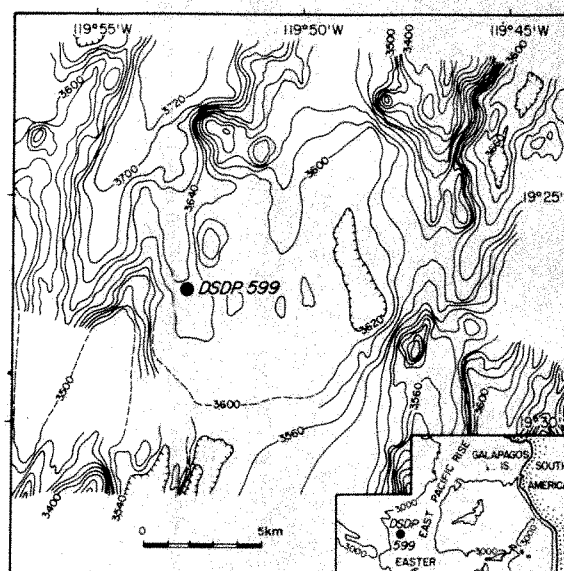


Fig. 1 Bathymetry (from seabeam swath-mapping⁴) and location of Deep Sea Drilling Site 599 on the west flank of the East Pacific Rise. This site, along with the other DSDP Leg 92 sites, was the first on the East Pacific Rise to be drilled using the hydraulic piston corer, which minimizes sediment disturbance during the coring process.

Downslope transport of metalliferous sediments along the East Pacific Rise during the late Miocene

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The distribution of metalliferous sediments next to active spreading centres has scientific and economic interest¹. Although metal-rich waters emanating from active hydrothermal vents have been traced in intermediate level water masses far beyond the ridge crest², the greatest concentrations of metal oxides in sediments occur near the vents³. There, however, it is conceivable that the oxides may be redistributed and possibly further concentrated by redeposition, resulting in misconceptions of the age and relative timing of hydrothermal pulses. Here, we document microfossil evidence of stratigraphical inversion and redeposition of Upper Miocene (Messinian) sediments cored at Deep Sea Drilling Project (DSDP) Site 599 on the East Pacific Rise (EPR). This suggests that where reworking can be confirmed, care should be taken not to correlate directly the occurrences of the metalliferous sediments with apparent coeval pulses of hydrothermal activity or enhanced seafloor spreading rates.

Hole 599 (19°27.09' S, 119°52.88' W; water depth = 3,654 m), located ~600 km west of the present ridge crest, was drilled in a small basin surrounded by a region of low relief with abyssal hills ranging from 200 to 300 m in height (Fig. 1). This site yielded some 41 m of mostly Upper Miocene clay-bearing to clayey calcareous oozes with a basement age of 8.1-8.6 Myr (ref. 5). The lower 31 m of the section is strikingly layered, consisting of alternating light (mostly yellowish brown to dark yellowish brown) and dark (mostly dark reddish brown) coloured layers tens of centimetres thick (Figs 2, 3). The colour variations are the result of changes in the relative amounts of calcium carbonate (70-80% in light coloured sediments, 55-70% in dark coloured sediments⁵) and a noncarbonate fraction which is primarily clays and ferruginous grains. The clays are mixtures of poorly crystalline smectites and amorphous oxides. The ferruginous grains, which range in size from 10 to 100 µm, are a metalliferous hydrothermal component which has been described by Leg 34 scientists as red-brown to yellow-brown, semi-opaque oxides (RSOs)⁶.

The strongly layered portion of the section is dated between

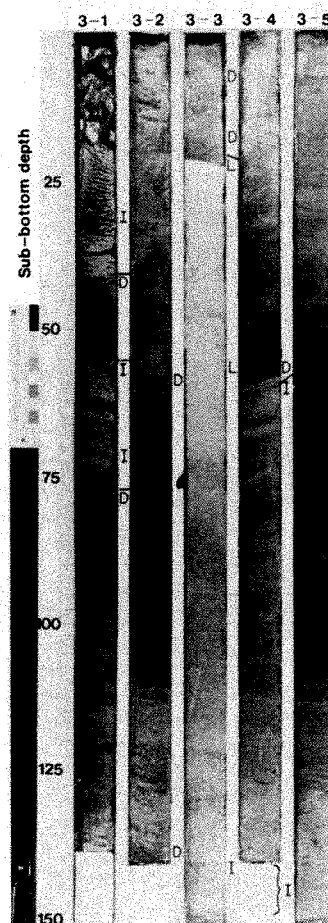


Fig. 2 Sections 1-5 of DSDP Core 599-3 showing alternations of light (L), dark (D), and intermediate (I) coloured lithologies within the Messinian clayey nannofossil ooze sequence (see key in Fig. 3 for detailed colour descriptions). The darker lithologies contain less carbonate and more hydrothermal metalliferous components than do the lighter lithologies. Note the sharp contact (dark over light) at 21 cm in section 599-3.

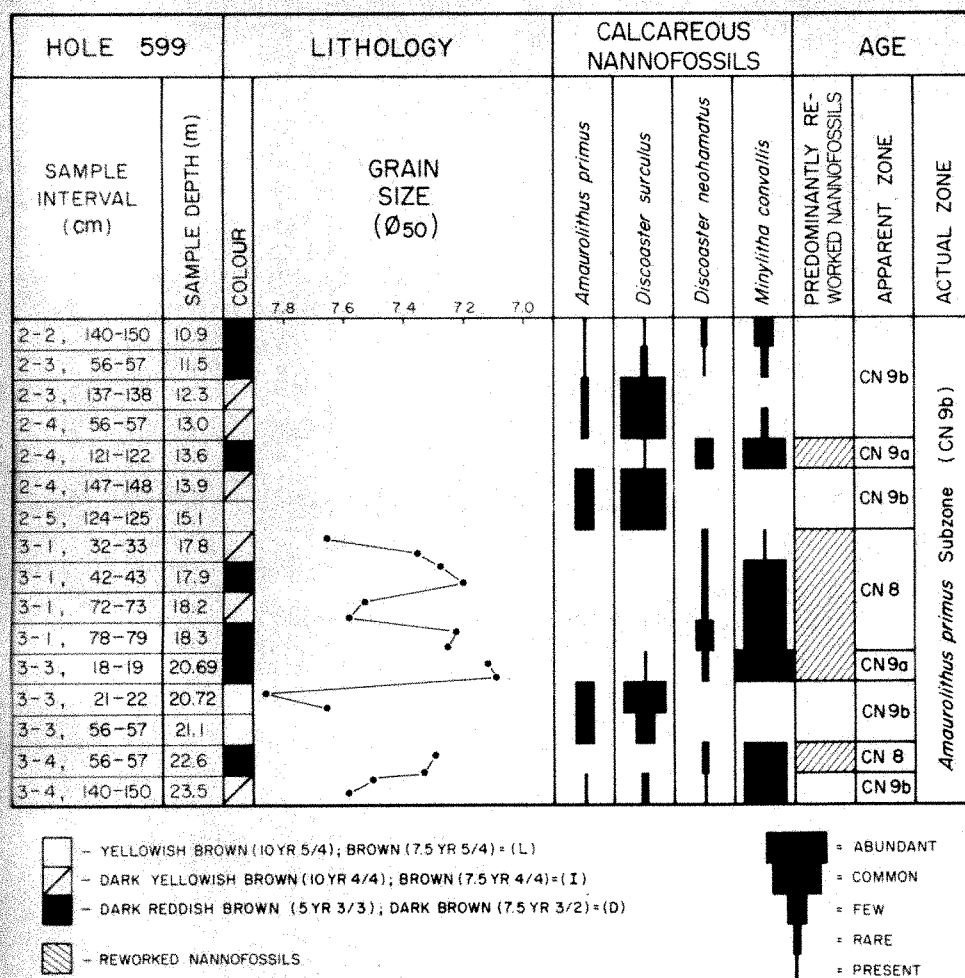


Fig. 3 Correlation of colour, grain size and calcareous nannofossil assemblages in a portion of the Messinian section at Site 599. The darker and coarser grained, redeposited metalliferous sediments contain reworked microfossil assemblages which indicate apparent ages up to 2.5-Myr older than the actual age of the sequence.

5.0 and 8.6 Myr (ref. 7) using the calcareous nannofossil zonation of Okada and Bukry⁸ as calibrated against time by Haq⁹. The bulk of that section (12.3–30.7 m), however, falls within the *Amaurolithus primus* Subzone (CN9b) of the *Discoaster quinqueramus* Zone which, according to Haq⁹, spans the Messinian Stage (6.7–5.4 Myr). That age determination is not immediately apparent, however, because this part of the section contains anomalous stratigraphical inversions of coccolith assemblages with older assemblages (represented in Fig. 3 by zones of lower numbers or letters) interbedded with the younger sediments of Subzone CN9b. The older assemblage of the 'apparent' Zone CN8 ('apparent age' = 9.2–8.1 Myr) is characterized by many *Minylitha convallis* and few *Discoaster neohamatus*, whereas the younger assemblage of Subzone CN9b is characterized by many *Discoaster surculus* and few *Amaurolithus primus*. The older assemblages are considered to be reworked and/or redeposited. There is little detectable mixing between these assemblages.

Detailed studies were carried out on Core 599-3 where the colour banding is pronounced (Fig. 2). As indicated in Fig. 3, there is a strong correlation between the occurrence of the older coccolith assemblages and the dark or intermediate-coloured metalliferous sediments⁷. There is also a strong correspondence between colour and grain size of the sediment, with the darker sediment containing coarser material¹⁰ (Fig. 3). These correlations are particularly evident at the sharp contact in Section 599-3-3 at 21 cm. We conclude that this contact marks the base of a turbidite which transported or concentrated sediments with an anomalously high content of metalliferous components into the basin. As indicated for other parts of the section depicted in Fig. 3, this process of redeposition could well account for much of the hydrothermal material deposited in this portion of the section recovered at Site 599.

The juxtaposed coccolith assemblages correlated above with

textural characteristics of the sediments, which include examples of graded bedding, provide strong evidence for downslope processes active on the EPR during the late Miocene. The timing of these mass movements correlates closely with the Messinian sea-level drop and intensified current activity which have been widely recorded in many ocean basins^{11–13}. Displaced shallow-water fossils of a similar origin are also recorded in many other tropical and sub-tropical Pacific DSDP sites^{14–16}, therefore the triggering mechanism for the turbidites at Site 599 may be related to erosion by intensified currents. An alternate explanation is tectonic instability associated with possible increased seismic activity along the EPR. This, in turn, might be coupled with accelerations in volcanic discharge and seafloor spreading rates which are thought to have peaked in the Pacific at ~5 Myr (ref. 17). These speculations are further elaborated in more extended discussions to be described elsewhere^{7,10}. By either mechanism, however, the concentration and distribution of the metalliferous sediments seem to be appreciably affected by processes of redeposition at this locality. Redistribution of these sediments was probably enhanced by their relatively low specific gravity and by the topographic relief at this site (Fig. 1). Should a similar configuration of strongly banded sediments with sharp basal contacts beneath the dark coloured layers be encountered elsewhere, redeposition might reasonably be suspected. If reworking and/or redeposition is suggested by palaeontological evidence, care should be taken not to correlate directly the increased concentration of metalliferous sediments with episodes of heightened hydrothermal activity or enhanced seafloor spreading rates.

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'Strangelove ocean' before the Cambrian explosion

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The Palaeozoic and Mesozoic eras were terminated by faunal changes involving mass extinction of the old and explosive evolution of the new fauna, but the fossil record shows only a Cambrian Explosion at the end of the Precambrian. Stanley speculated that the explosion was only possible after the ubiquitous algae community had been largely eliminated¹; ecological niches were thus liberated for explosive evolution. If the Cambrian Explosion were preceded by a mass mortality (or by a mass extinction), such an event should leave a record in the form of geochemical anomalies. We have undertaken a search for geochemical anomalies at the Precambrian/Cambrian contact. We report here the discovery of a sharp negative carbon-isotope shift in the carbonate of a clay immediately above a marker in the Precambrian/Cambrian boundary, the China C marker, and interpret this signal as evidence of sudden decrease in fertility before the Cambrian explosion of invertebrate evolution. The discovery suggests that the Precambrian/Cambrian boundary might be defined by an event-marker at a palaeontologically correlative horizon.

Investigations of the Cretaceous/Tertiary boundary reveal that the boundary event left a signature in an iridium anomaly and a carbon isotope anomaly^{2,3}. The dual Ir- $\delta^{13}\text{C}$ anomalies were also found recently in a Permian/Triassic boundary clay⁴. The exact placement of Precambrian/Cambrian boundary has not yet been established. Chinese scientists have suggested three datum levels as candidates: China A marks the first appearance of inarticulate brachiopods and China B defines the base of the *Paragloborilu Siphonogonuchites* Zone, characterized by a more diversified shelly fauna. The third, or the China C marker, is slightly above the China B (Fig. 1), and defined by a remarkable lithological change from a phosphate-rich rock to a black shale. This horizon marks the base of a formation containing the first trilobites⁵, and has been correlated for thousands of kilometres across South China.

Following the discovery in 1982 of an iridium anomaly in a black shale above the China C marker (C. C. Woo, personal communication) one of us (K.J.H.) took samples across the iridium-rich horizon. Six samples from a 12-m section were obtained at the Type Locality for the Precambrian Sinian Group in the Yangtze Gorge section, ~20 km west of Ichang, Hupeh Province, China. Analysis confirmed the presence of higher

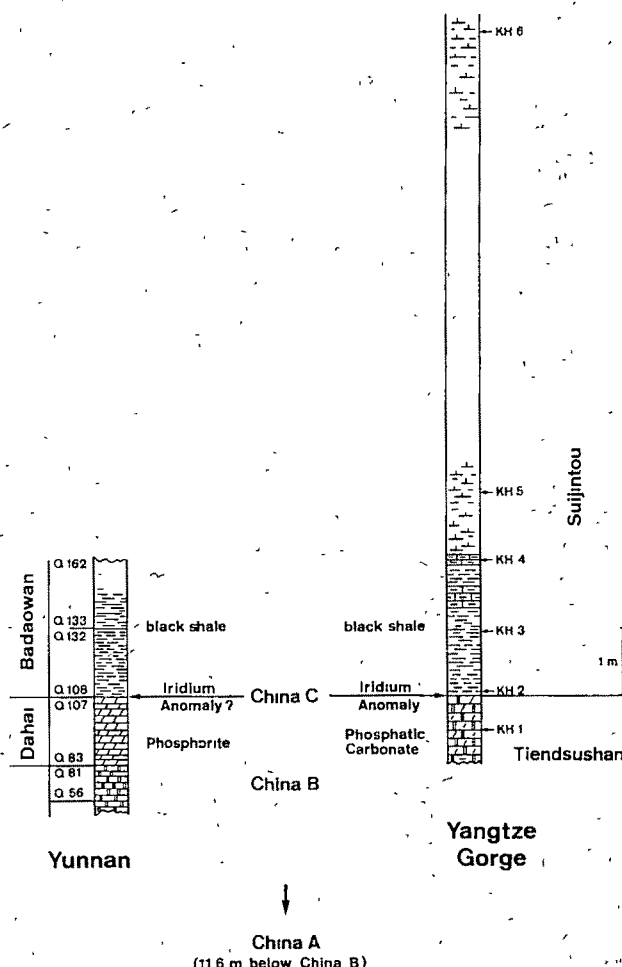


Fig. 1 Stratigraphical columns across a proposed Precambrian/Cambrian contact (China C marker) at two localities in south-west China. Iridium anomaly has been identified in the boundary clay at the Yangtze Gorge locality. The Q and KH numbers are sampling numbers. The Q samples have been obtained at 2-cm intervals apart. KH samples were taken for a reconnaissance study. Not shown are the densely spaced samples from the Yangtze locality. China B marks the base of Dahai.

iridium and osmium contents in a black clay immediately above the China C marker. Also found was a carbon-isotope anomaly at the iridium-enriched horizon (Fig. 2).

The discovery prompted us to resample across the China C marker in 1984 to produce a more systematic investigation. Fifty-three samples were taken from the Yangtze Gorge locality near the marker at intervals spaced 2-cm apart. Some 100 samples were taken from the Kunyang Mine with 2-cm sampling intervals, not only across China C, but also across China A and China B markers. The close sampling was done to define precisely the carbonate-isotope anomaly, and to determine whether the anomaly is a signal or a random variation. The stratigraphy of both localities has been described in detail elsewhere^{5,6}. Note that the first trilobite specimens were collected in a calcareous shale within 1 m above the China C marker at the Yangtze locality, but at a horizon some 10 m higher at Kunyang.

The isotope analyses were carried out at ETH Zurich. The phosphatic rocks contain 90% carbonate and the black shale contain between 8 and 26% carbonate, which is mainly dolomite. A thin seam of black shale immediately above the China C marker can, however, be considered a boundary clay layer; the sample at the Kunyang Mine contains 0-4% carbonate, and the one at the Yangtze Gorge locality is practically carbonate-free, thus yielding no isotope results.

The $\delta^{13}\text{C}$ values of the dolomite at the two localities are shown by Fig. 3. The background values are slightly more negative at

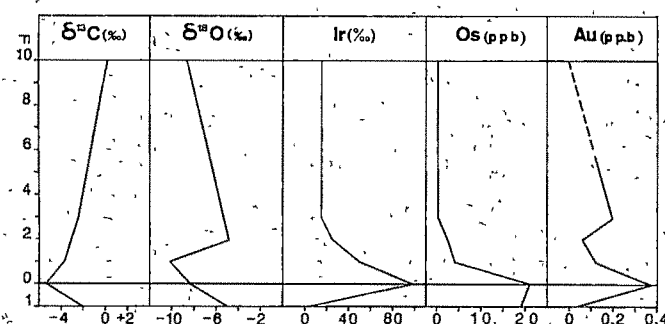


Fig. 2 Geochemical anomalies across the China C marker at Yangtze locality.

the Yangtze Gorge than those at the Kunyang locality. Note the sharp anomaly of $\sim 5\%$ more negative values exactly at a horizon immediately above the China C marker. This anomaly is present at both localities, which are $\sim 1,000$ km apart.

The sedimentation rate of the Cambrian black shale cannot be accurately determined. Chinese scientists estimated an average sedimentation rate of <10 m Myr^{-1} , for the Cambrian rocks, based on the thickness of the Cambrian strata ($<1,000$ m) of the Yangtze Gorge Section⁶ and the duration of the Cambrian Period (>100 Myr). Assuming such a rate, the isotope shift at the China C datum should have taken place within 2,000 yr. The anomaly is thus a sharp perturbation, comparable in its magnitude and sharpness with those found across the Cretaceous/Tertiary and Permian/Triassic boundaries.

Samples across the China A and China B markers at Kunyang are still being analysed. We have not yet found iridium nor carbon-isotope anomalies across those boundaries.

The values of $\delta^{13}\text{C}$ in marine carbonate is influenced by many factors, such as terrestrial influx, ocean-atmosphere exchange, and biological productivity⁷. Thousands of analyses of deep-sea drill cores indicate that carbonate-isotope values of ocean sediments do not vary rapidly with time, on account of the very large reservoir of dissolved carbonate atoms in ocean waters. No sharp global anomalies ($2\text{--}4\%$ in 10^3 or 10^4 yr) have ever been observed except across the Cretaceous/Tertiary boundary, although long-term variations with anomaly values up to 4% over a $10^6\text{--}10^7$ -yr period are not uncommon.

Sharp perturbation of ^{13}C values in sediments has been related to ocean fertility. Natural fractionation of carbon isotopes by biological activity is well known. Carbon-12 atoms are preferentially used by living organisms, so that the carbon-13 atoms in dissolved carbonates are enriched in surface waters where the planktons flourish. This phenomenon has been confirmed by isotope analyses of dissolved carbonates in ocean and lake waters^{7,8}. Sudden changes in biological productivity produce $\delta^{13}\text{C}$ anomalies in surface water. Two types of anomalies have been recognized, namely a positive anomaly of 'fertility perturbation' and a negative anomaly or 'Strangelove perturbation'.

The 'fertility perturbation' was discovered by McKenzie at Lake Greifen⁹. There was a positive anomaly of 4.5% in the inorganic $\delta^{13}\text{C}$ in the surface waters during late July and August (months of high fertility, when green algae production reaches a maximum) (Fig. 2). No such anomaly was detected in the inorganic carbon of the bottom sediments. In fact, a slight negative anomaly of $<1\%$ was detected in September, the month after the harvest, when the light carbon in bottom waters was relatively enriched because of the decay of dead organisms.

The exact opposite type of perturbation has been found at Cretaceous/Tertiary and Permian/Triassic boundaries^{3,4}. In ancient as in modern ocean waters, the inorganic carbon of surface waters is normally enriched in carbon-13. The $\delta^{13}\text{C}$ is $\sim 2\%$, more positive than that of bottom waters because of the steady production of plankton. Fertility is a normal state. After events such as those marking era-boundaries, the environmental pollution may have been such as to reduce or to suppress

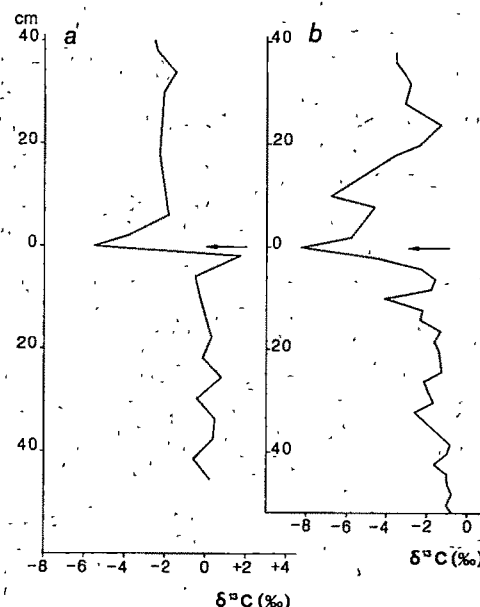


Fig. 3 Strangelove perturbation across the China C marker. a, Kunyang Mine locality, Yunnan Province. b, Yangtze Gorge locality.

plankton production for thousands of years. This hypothetical state of ocean, almost devoid of living organisms, has been called the 'Strangelove Ocean' (refs 8, 9). The geochemical expression of this ocean is a perturbation in the $\delta^{13}\text{C}$ value of planktonic sediments: when the carbon-isotope fractionation by biological activity ceases, a mixing of surface and bottom waters should produce a carbon-isotope composition that is consistent from the top to the bottom of the water column. Consequently, the planktonic sediments deposited during a crisis should have a $\delta^{13}\text{C}$ value similar to that in the bottom sediments, and be considerably more negative than the planktonic sediments deposited in normally fertile conditions. The presence of a Strangelove perturbation across the Cretaceous/Tertiary boundary is thus considered to be evidence of an interrupted plankton production following a boundary event⁹.

Calcareous planktons, such as foraminifera and nannoplanktons, had not yet evolved during the Precambrian. Yet Precam-

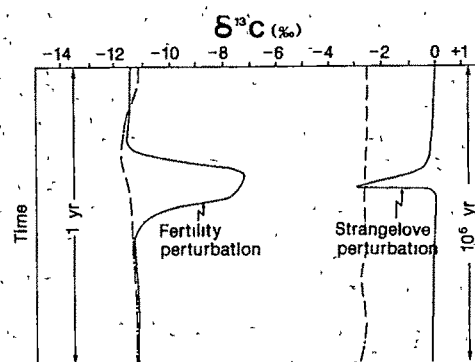


Fig. 4 Carbon-isotope anomaly related to plankton productivity. The left-hand side shows the annual variation of carbon-isotope values in dissolved carbonates of surface water (solid line) and of bottom water (dashed line) in Lake of Greifen, Switzerland. Time at the bottom is January and at the top is December. A positive perturbation in surface water is observed in July and August when the plankton fertility reaches the maximum. The right-hand side of the figure is the short-term perturbation of carbon-isotope values across the Cretaceous/Tertiary boundary at DSDP Site 524 in the South Atlantic Ocean, showing the changes in the planktonic (solid line) and in the benthic (dashed line) foraminifera. The base of the negative Strangelove perturbation coincides with the Cretaceous/Tertiary boundary, marked by an iridium anomaly; and the perturbation is believed to indicate that the biomass of ocean planktons had been drastically reduced.

brian pelagic or hemipelagic sediments contain carbonates. The carbonates were probably produced through the biological activity of planktonic organisms, such as phytoplanktons or green algae. These left no fossil skeletons, but their photosynthesis removed CO_2 from the surface water to cause carbonate oversaturation and precipitation. This process of calcite production occurs annually in the Swiss lakes¹⁰. If a catastrophe should have occurred, and resulted in suppressed plankton production in Precambrian oceans, the biological fractionation of carbon isotopes would cease. Consequently, we could interpret the large negative $\delta^{13}\text{C}$ anomaly at the 'China C datum' as a Strangelove perturbation. Geochemical anomalies thus support the theory¹ that early Cambrian evolution of multicellular life was preceded by a catastrophic destruction of the late Precambrian biomass.

Some unknown single-celled herbivores were held responsible for the destruction of the ubiquitous algal community of the Precambrian¹. The idea is not supported by palaeontological evidence, because the postulated culprit left no fossil skeletons. Furthermore, the Cambrian explosion was preceded by the evolution and extinction of the Ediacarian soft-bodied fauna¹², which could not have been vulnerable to the herbivores in ref. 1. If there was a major extinction before the Cambrian explosion, some other cause must be sought.

Note that an iridium anomaly in the black shale immediately above the China C marker has been found in many parts of South China^{12,13}. The anomalously high values up to 30 p.p.b. (parts per 10^9) Ir (ref. 12) in metalliferous sediments may have resulted from secondary enrichment, but the enrichment may have been preceded by a primary source of iridium. The possibil-

ity of a large-body impact before the Cambrian explosion must thus be considered an attractive working hypothesis.

The dual iridium $\delta^{13}\text{C}$ anomaly in a boundary clay above a palaeontological datum has only been found globally across the Cretaceous/Tertiary boundary and, in China, across the Permian/Triassic boundary. The presence of the same association of an iridium-anomaly/Strangelove-perturbation/boundary-clay above a palaeontological datum marking the first appearance of trilobites cannot be coincidental. Whatever the ultimate cause of the geochemical anomalies, the possibility that the Precambrian/Cambrian boundary is definable by an event marker is worthy of further exploration. The IGCP Project 199 on Rare Events in Geology has undertaken the task of international cooperation to ascertain if geochemical anomalies similar to those reported here can be found elsewhere.

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Mass extinction among non-marine tetrapods

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The fossil record of non-marine tetrapods (amphibians, reptiles, birds and mammals) has been described by numerous authors¹⁻³, and major ecological replacements, mass extinctions and adaptive radiations have been identified. However, most of these features of the large-scale evolution of tetrapods have been noted without numerical data of the kind assembled for marine invertebrates⁴⁻¹⁰, marine vertebrates⁷⁻¹⁰ and vascular land plants¹¹. Much has been learnt from the record of marine invertebrates, particularly about the overall patterns of diversification with time, the performance of different major taxonomic groups at different times, and the magnitude and timing of mass extinctions. The present study tests some of the general conclusions on the basis of a new compilation of data on the fossil record of terrestrial tetrapods. I show that family diversity rose with time, and in particular from the Cretaceous to the present day. There were several mass extinction events, but none of these was associated with a statistically high extinction rate. The extinction events, including the famous terminal Cretaceous extinction, were the result of a slightly elevated extinction rate combined with a depressed origination rate, and the present evidence does not support the view that mass extinctions are statistically distinguishable from background extinctions. Further, the record of non-marine tetrapods shows an increasing total extinction rate and an only marginally decreasing probability of extinction (per-taxon rate) from the late Devonian to the present, the opposite of the findings from the record of marine animals.

The present study is concerned with the fossil record of non-marine tetrapods: this includes terrestrial, freshwater and flying forms, but excludes fully marine families. A list of all the families of terrestrial tetrapods was made using the most recent taxonomic reviews, as well as secondary literature, to the end of 1984 (for details see ref. 12). A small number of extinct

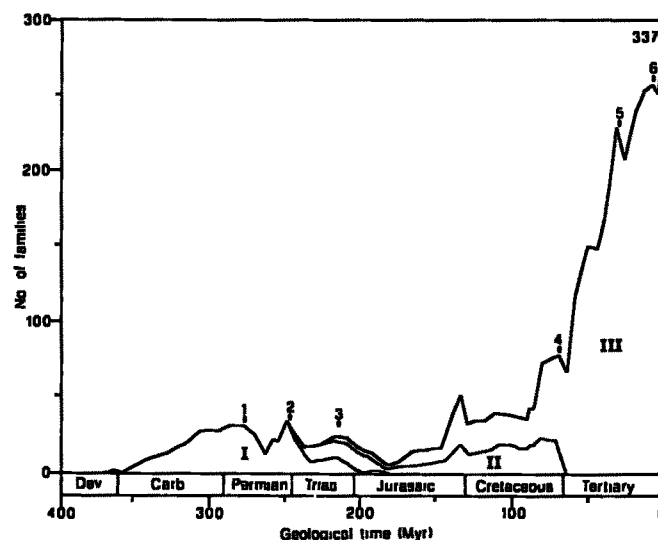


Fig. 1 Standing diversity with time for families of terrestrial tetrapods. The upper curve shows total diversity with time, and six apparent mass extinctions are indicated by drops in diversity, numbered 1-6. The relative magnitude of each drop is given in terms of the percentage of families that disappeared: (1) early Permian (Sakmarian-Artinskian), 58%; (2) late Permian-early Triassic (Tatarian-Scythian), 49%; (3) late Triassic (Norian-Rhaetian), 28% (4) late Cretaceous (Maastrichtian), 14%, (5) early Oligocene (Rupelian), 8%; late Miocene (Tortonion-Messinian), 2%. The mass extinctions were produced by a combination of a slightly elevated extinction rate and a reduced origination rate. The timescale used was that of Palmer¹⁶. Three assemblages of families succeeded each other through geological time: I, labyrinthodont amphibians, anapsids, mammal-like reptiles; II, early diapsids, dinosaurs, pterosaurs; III, the 'modern' groups: frogs, salamanders, lizards, snakes, turtles, crocodiles, birds, mammals. These assemblages were defined somewhat arbitrarily to encompass those groups that dominated non-marine tetrapod faunas in the late Palaeozoic, the Mesozoic and the Cenozoic respectively. The data are not robust enough for the application of the factor analytic technique of Sepkoski⁶. Abbreviations Carb., Carboniferous; Dev., Devonian, Trias., Triassic.

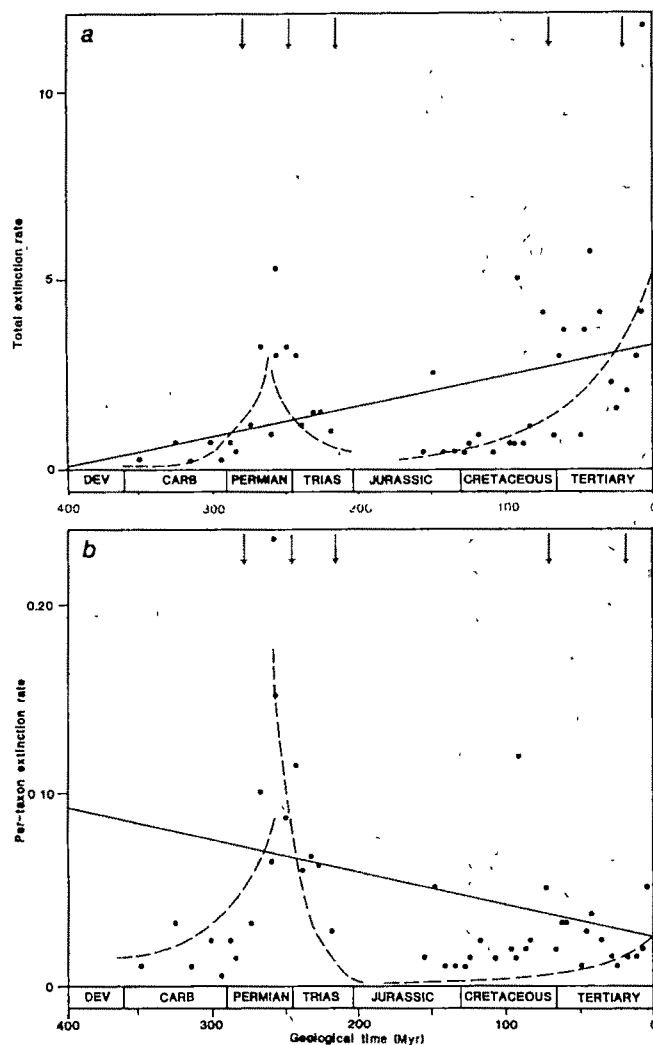


Fig. 2 Total extinction rate (a) and per-taxon extinction rate (probability of extinction) (b) for families of terrestrial tetrapods. The total extinction rate increases with time according to the linear regression $y = 0.00832x + 3.497$ ($P = 0.003$; $r^2 = 0.16$), while the per-taxon extinction rate declines with time according to the linear regression $y = -0.00013x + 0.017$ ($P = 0.024$; $r^2 = 0.09$). The extinction rate data in both cases are better explained by three log linear regressions for different time divisions (these are indicated by dashed curves; see Table 1 for the equations). Extinction rates were calculated for 45 stages between the early Carboniferous and the Pleistocene: the late Devonian (Famennian) and early to middle Jurassic stages (Hettangian–Oxfordian) were excluded because of the poor fossil record within these intervals. The episodes of mass extinction determined in Fig. 1 are indicated by arrows. Abbreviations as in Fig. 1.

families were excluded from the analysis as they were based on single, often incomplete, specimens; this left a total of 730 families, of which 469 are extinct. The quality of the fossil record of terrestrial tetrapods is variable because certain kinds of environments are poorly preserved, and there is evidence that the record is particularly bad in the early and middle Jurassic, and in the mid-Cretaceous (see below). The range in geological time for each family was determined from the most recent available literature, and this was resolved to the level of the stratigraphic stage (duration 2–19 Myr; mean duration 6 Myr).

The numbers of families of terrestrial tetrapods are plotted against geological time in Fig. 1. The most striking feature of this graph is that the number of families present at any time remained at levels below about 50 until the last quarter of the Cretaceous period when the numbers of families increased to nearly 80, and the rise continued to the present total of 337 in several steps during the Tertiary. This great increase in family diversity may be partly an artefact of the fossil record which

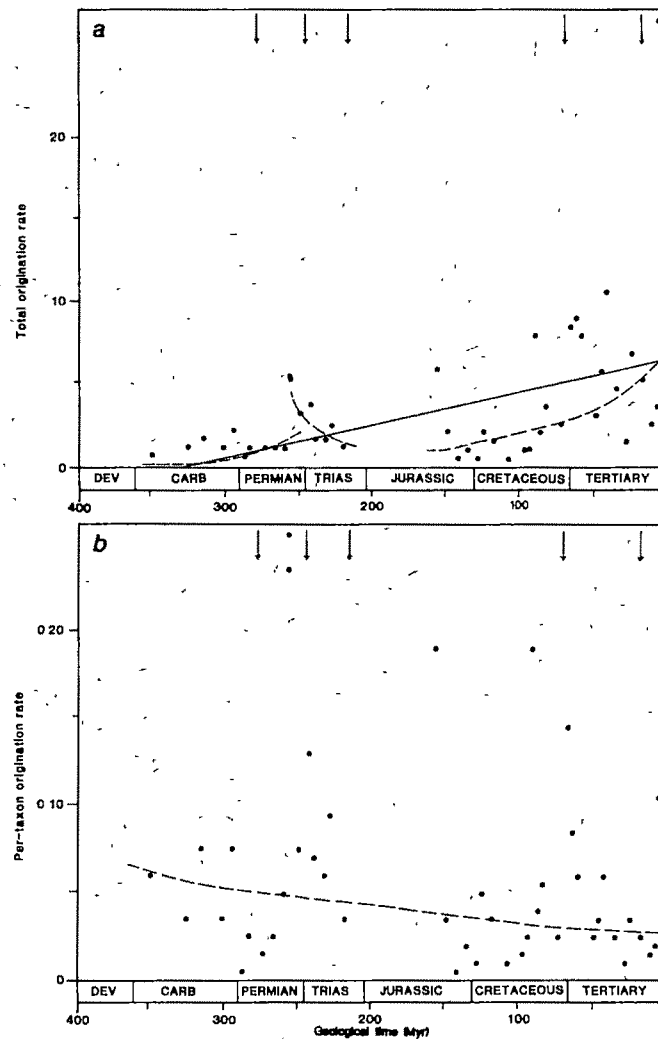


Fig. 3 Total origination rate (a) and per-taxon origination rate (probability of origination) (b) for families of terrestrial tetrapods. The total origination rate increases with time according to the linear regression $y = 0.02008x + 6.356$ ($P = 0.001$, $r^2 = 0.21$), while the per-taxon rate declines with time according to the log linear regression, $\log y = 0.00105x - 1.582$ ($P = 0.048$, $r^2 = 0.07$). The total origination rate data are better explained by three log linear regressions for different time divisions (these are indicated by dashed curves; see Table 2 for equations). The per-taxon origination rate data did not correspond to a statistically significant linear regression, nor were significant regressions derived for different time divisions: only the late Permian to late Triassic data fell along a significant linear or log linear regression (for the equations, see Table 2). Other information is given in Fig. 2 legend.

generally improves towards the present, and of the greater research emphasis devoted to Tertiary fossil vertebrates than to earlier ones. However, a large part of the increase is probably a real biological phenomenon, as has been shown for the record of marine invertebrates.^{13,14}

The second interesting feature of this analysis is that it shows a succession of family assemblages that dominated for a time and were then replaced (Fig. 1). The first assemblage of tetrapod families—labyrinthodont amphibians, anapsids and mammal-like reptiles—dominated from the late Devonian to the early Triassic. The second assemblage—primitive diapsid reptiles, thecodontians, dinosaurs and pterosaurs—arose in the Permian and increased in diversity through the Triassic as the members of the first assemblage declined. This second assemblage dominated from the late Triassic to the late Cretaceous. The third assemblage, which includes all modern tetrapod groups—frogs, salamanders, lizards, snakes, turtles, crocodiles, birds and mammals—arose in the late Triassic, and increased in diversity

through the Jurassic and early Cretaceous. During the Cretaceous, the diversity of these modern groups was approximately equal to that of the second assemblage of dinosaurs and pterosaurs. In the latest Cretaceous, the diversity of the modern groups rose dramatically and became twice that of the dinosaurs and pterosaurs, long before the terminal Cretaceous extinction event. The modern groups were barely affected by this extinction, and their family diversity increased through the Tertiary. Members of the first assemblage reached an observed familial diversity of about 30 in the Permian, whereas dinosaur and pterosaur families reached an observed diversity of only 20–25 families in the Cretaceous.

The third part of this study concerns the occurrence of mass extinctions in the fossil record of terrestrial tetrapods. The terminal Cretaceous event, during which the dinosaurs and pterosaurs disappeared, is well known^{15–17}, and other extinctions among tetrapods have been suggested in the middle of the Permian^{18,19}, at the end of the Permian^{18,19}, at the end of the early Triassic^{18,19}, and at the end of the Triassic^{18–20}. A mass extinction may be identified at first by the observation of a drop in diversity. The general upward trend of the plot of family diversity of terrestrial tetrapods with time (Fig. 1) is interrupted by several major reductions in family diversity, in the early to mid-Permian (Sakmarian–Artinskian), the late Permian to early Triassic (Tatarian–Scythian), the late Triassic to early Jurassic (Norian–Hettangian), the late Jurassic (Portlandian), the late Cretaceous (Maastrichtian), the early Oligocene (Rupelian) and the late Miocene (Tortonian–Messinian).

These episodes of diversity decrease may not all represent mass extinctions. Two of them (early Jurassic, late Jurassic) are followed by times during which there is a very poor fossil record of terrestrial tetrapods (that is, there are no fossils known, or only one or two, from a particular stratigraphic stage). In these two cases, much of the decline in diversity, if not all of it, could be the result of the poor fossil record, and the conservative conclusion is to assume that these two are not mass extinctions. The initial part of the late Triassic to early Jurassic mass extinction began well before the poor fossil record of the early to middle Jurassic, and the earlier part is retained as an extinction event¹². The mid-Permian decline in diversity might also partially reflect a gap in the fossil record, but, again, at least the earlier part is probably a mass extinction.

The six remaining declines in diversity are listed in Fig. 1 as a percentage of the total number of families present at the start. These vary in magnitude from 2% in the late Miocene to 58% at the end of the early Permian. The first three extinctions, in the Permian and Triassic, had the greatest overall effects (falls of 28–58%), but each of these lasted over two stratigraphic stages (durations of 14, 13 and 17 Myr for extinctions 1–3 respectively). The terminal Cretaceous event had a lower overall effect (drop of 14%), but it occurred in a single stratigraphic stage (duration 8 Myr). The two Tertiary extinctions (numbers 5 and 6) had much smaller relative effects on overall diversity.

A decline in family diversity could be the result of a high extinction rate, or a low origination rate, or a combination of the two^{18,21}. It is commonly assumed that declines in diversity (mass extinctions) are caused only by a high extinction rate, and reasons are then sought to explain the 'dying off'. However, if the decline in diversity were associated with a depressed family origination rate, and the extinction rate was normal, we would have to seek other kinds of explanations.

The nature of the six terrestrial tetrapod mass extinctions was explored by an analysis of the total extinction and origination rates (simply the numbers of families dying out or appearing during a stage, divided by the duration of that stage), and the probabilities of extinction or origination (calculated for each stage as the total rate divided by the number of taxa 'at risk' or 'available', according to Van Valen's²² definition; also called per-taxon rates).

First, the total and per-taxon rates were plotted against geological time (Fig. 2) to determine whether the times of observed mass extinctions were marked by high extinction rates. Most

Table 1 Regression equations for total rates and per-taxon rates (probabilities) of extinction for non-marine tetrapods from the late Devonian to the present

	<i>P</i>	<i>r</i> ²
a Total rates		
(1) (All stages) $y = 0.00832x + 3.194$	0.003	0.16
(2) (All stages) $\sqrt{y} = 0.00301x + 1.650$	0.001	0.20
(3a) (Kimm-Pleist) $\log y = 0.00841x + 0.698$	0.000	0.53
(3b) (Kaz-Nor) $y = -0.06644x - 13.903$	0.007	0.73
(3c) (Kaz-Nor) $\log_{10} y = -0.01652x - 3.709$	0.005	0.76
(3d) (Tour-Ufim) $\log_{10} y = 0.01872x + 5.153$	0.001	0.75
b Per-taxon rates (probabilities)		
(4) (All stages) $y = -0.00013x + 0.017$	0.024	0.09
(5a) (Kimm-Pleist) $\log_{10} y = 0.00138x - 1.729$	0.182	0.03*
(5b) (Kaz-Nor) $y = -0.00275x - 0.573$	0.005	0.76
(5c) (Kaz-Nor) $\log_{10} y = -0.01666x - 5.099$	0.002	0.84
(5d) (Tour-Ufim) $\log_{10} y = 0.01247x + 2.054$	0.006	0.56

Probabilities of extinction were calculated using the Van Valen²² metric (that is, the number of extinctions (last appearances) in a stage, divided by the number of taxa at risk. The latter was estimated as the number of taxa at the beginning of the interval, minus half the extinctions, plus half the originations in the interval). The equations that give the highest percentage of explained sum of squares (*r*²) are listed here. Equations are given for all stages treated together, and then for a three-way division of the data, with splits in the mid-Permian (Ufim/Kaz) and in the early to middle Jurassic (Nor/Kimm). Note the absence of data for the stages of the early and middle Jurassic. The probability (*P*) of the correctness of each regression equation is also given; Kaz, Kazanian; Kimm, Kimmeridgian; Nor, Norian; Pleist, Pleistocene; Tour, Tournaisian, Ufim, Ufimian.

* *P* > 0.1 (nonsignificant results) are marked by an asterisk.

total extinction rates fell below 4 per Myr, but several rates were higher (up to 12 family extinctions per Myr), while most probabilities of extinction fell below 0.120 per family per Myr, with higher rates up to 0.240. Both graphs gave highly significant linear regressions (*P* = 0.003, *P* = 0.024; Table 1). Raup and Sepkoski⁷ suggested that mass extinction events were associated with statistically very high extinction rates (outliers above the 95% confidence envelope of a linear regression of all extinction rates), but this technique did not work for the non-marine tetrapod record. Most of the outliers (that is, stages during which extinction rates were statistically very high, for example, Pleistocene, Ufimian), did not correspond to falls in overall diversity, as they were matched by even higher origination rates. The technique also did not work when the extinction data were transformed to logarithms²³ or to square roots²⁴.

The total and per-taxon origination rates were then plotted against geological time (Fig. 3) to determine whether the times of observed mass extinctions were marked by statistically low origination rates. The graph of total origination rates (Fig. 3a) gave a highly significant linear regression (*P* = 0.001), but the graph of probabilities (Fig. 3b) did not (Table 2). Statistical tests for outliers—extremely low origination rates—did not pick out the times of mass extinction. The statistically low rates did not correspond to falls in diversity because they were matched by high extinction rates.

Thus there is no evidence in the record of non-marine tetrapods that mass extinctions are statistically distinguishable from 'background' extinctions, by having either a very high family extinction rate or a very low family origination rate. Note, however, that the time resolution in this study, as in most others

Table 2 Regression equations for total rates and per-taxon rates (probabilities) of origination for non-marine tetrapods from the late Devonian to the present

a Total rates	P	r ²
(1) (All stages) $y = 0.02008x + 6.356$	0.001	0.21
(2) (All stages) $\log_{10} y = 0.00254x + 0.636$	0.000	0.28
(3a) (Kimm-Pleist) $\sqrt{y} = 0.01333x + 2.790$	0.001	0.31
(3b) (Kimm-Pleist) $\log_{10} y = 0.00629x + 0.849$	0.002	0.29
(3c) (Kaz-Nor) $\log_{10} y = 0.01804x - 4.008$	0.009	0.71
(3d) (Tour-Ufim) $\log_{10} y = 0.00580x + 1.556$	0.085	0.22
b Per-taxon rates (probabilities)		
(4) (All stages) $y = -0.00010x + 0.042$	0.137	0.03*
(5) (All stages) $\log_{10} y = -0.00105x - 1.582$	0.048	0.07
(6a) (Kimm-Pleist) $y = -0.00013x + 0.038$	0.286	0.01*
(6b) (Kimm-Pleist) $\log_{10} y = -0.00084x - 1.584$	0.337	0.01*
(6c) (Kaz-Nor) $y = -0.00425x - 0.906$	0.035	0.51
(6d) (Kaz-Nor) $\log_{10} y = -0.01816x - 5.394$	0.017	0.63
(6e) (Tour-Ufim) $y = 0.00052x + 0.204$	0.247	0.05*
(6f) (Tour-Ufim) $\log_{10} y = -0.00038x - 1.517$	0.468	0.00*

Probabilities of origination were calculated using an analogue of Van Valen's²² extinction probability metric. This, and other conventions and abbreviations, are explained in the legend to Table 1

published so far, is very coarse, and this could minimize the true size of a sudden event by averaging it out over several million years. In all cases, the declines in diversity (mass extinctions) were produced by slightly elevated extinction rates and slightly depressed origination rates. However, neither the total rates nor the probabilities were obviously different from 'normal' rates, and this statistical study points to a model of extinction events that does not distinguish mass extinctions as a discrete class of phenomena from background extinctions.

The graph of total extinction rates (Fig. 2a) shows that there was an overall rising trend with time. Most of this rise is explained by the increase in overall diversity (the more families there were, the more could become extinct in a unit of time). However, the probability of extinction (Fig. 2b) decreased with time, although by only a very small amount (0.00012 less families dying out per family per Myr; ~0.07% decline per stage). The increase in total extinction rates, and the small decrease in probability of extinction are very surprising, as the record of marine animals shows an overall markedly declining trend in both total and per-taxon extinction rates, the latter trend giving a decline of ~6.6% per stage^{7,22,25}. The graph of total origination rate (Fig. 3a) shows a rising trend with time, while the graph of probability of origination (Fig. 3b) did not yield a significant regression.

An attempt was made to find statistical models that described the data for extinction and origination rates more precisely (that is, giving a higher percentage of explained sum of squares, r^2), by splitting the data into several time intervals, and by transforming the rates into logarithms and square roots. The data set was not large enough, after refinement, for time series analysis^{10,25}, but it was found that the extinction rate data could be better described if the data were divided into three time sets: from the early Carboniferous (Tournaisian) to the mid-Permian (Ufimian), from the late Permian (Kazanian) to the late Triassic (Norian), and from the late Jurassic (Kimmeridgian) to the

Pleistocene. A set of three log linear regressions gave the best explanation of the total extinction rate data (Table 1, equations 3a-d), and the same applied to the per-taxon extinction rate data, although the third time interval was still poorly explained (Table 1, equations 5a-d). For total origination rates, three log linear regressions also provided the best model (Table 2, equations 3a-d), but the per-taxon origination rate data could not be so readily accounted for (Table 2, equations 6a-f). The best-fit regressions divide the data into three time segments (Figs 2, 3): (1) early Carboniferous to mid-Permian (rates rise sharply); (2) late Permian to late Triassic (rates fall sharply); (3) late Jurassic to Pleistocene (rates rise again).

The present analysis of the fossil record of non-marine tetrapods has shown two main points. First, none of the observed mass extinctions was caused by either very high rates of extinction or very low rates of origination. In fact, each event was associated with rates that did not differ statistically from normal background rates. Second, the total rate of extinction has risen with time from the late Devonian to the present day, and the probability of extinction has declined only marginally. The tetrapod record shows little evidence for overall improvements in the ability of organisms to 'resist' extinction—increases in darwinian fitness²².

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Susceptibility to murine lymphocytic choriomeningitis maps to class I MHC genes—a model for MHC/disease associations

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Susceptibility to some human diseases is linked, albeit weakly, to major transplantation antigens (HLA) encoded by the major histocompatibility gene complex (MHC)^{1,2}. Here we have studied MHC/disease association in inbred strains of mice after intracerebral (i.c.) injection of lymphocytic choriomeningitis virus (LCMV)³⁻⁶. This route of infection leads to a lymphocytic choriomeningitis (LCM) which is not the result of direct cytopathic effects of the virus but is caused by the induced T-cell immune response: immunocompetent mice die whereas T-cell-deficient mice

survive³⁻⁶. By using two plaque variants of LCMV strain UBC (refs 7, 8), we found that susceptibility to LCM was dependent on the LCMV strain used ('aggressive' versus 'docile' UBC-LCMV) and on the various genes of the host mouse strains. In addition, susceptibility to LCM caused by docile UBC-LCMV was clearly linked to the murine major histocompatibility locus *H-2D*: in MHC-congenic C57BL/10 mice, susceptibility correlated with early onset and high activity of measurable LCMV-specific cytotoxic T cells in meninges and spleens and could be mapped to *H-2D*. This model shows that a severe immunopathologically mediated clinical disease in mice can be regulated directly by MHC genes of class I type and supports the notion that many MHC/disease associations directly reflect MHC-restricted and MHC-regulated T-cell reactivity⁹.

Mice of various strains and various *H-2* types were assayed for susceptibility to docile and aggressive UBC-LCMV injected i.c. at >8 weeks of age (Table 1). Susceptibility to LCMV depended on the general genetic background of the injected mice: Swiss and a few B10 mice were susceptible to both UBC-LCMV substrains; most B10 and BALB mice were susceptible to aggressive but resistant to docile LCMV; C3H and CBA mice were susceptible to docile and relatively resistant to aggressive LCMV. In B10 mice, susceptibility to LCM caused by docile UBC-LCMV was found to be regulated by *H-2*; B10.G mice with the *q* haplotype, including (B10.BR × B10.G) (*H-2^k* × *H-2^q*)_{F1}, were susceptible in a dominant fashion, whereas mice of haplotype *H-2^k*, *H-2^d*, *H-2^b* and *H-2^a* and most others, were resistant. *H-2*-dependence of susceptibility to LCMV was also seen in DBA mice. DBA/1 mice (*H-2^q*) were susceptible to both strains whereas *H-2* congenic DBA/2 with *H-2^d* were resistant to both aggressive and docile LCMV. In C3H mice, *H-2* did not significantly influence susceptibility independent of *H-2^q* or *H-2^k*. Swiss inbred SWR/J (*H-2^q*)¹⁰, were highly susceptible to both UBC-LCMV strains. Thus, susceptibility to LCM depends on the virus variant injected and on the multiple genes of the host that influence resistance or susceptibility (such as interferon and macrophage resistance^{11,12}), only one gene being encoded in the MHC.

The role of various *H-2* regions in susceptibility to LCM was tested in several MHC-congenic inbred strains of mice with the B10 background (Fig. 1). B10.G(*H-2^q*: *K^q*, *I^q*, *D^q*), B10.AQR(*H-2^q*: *K^q*, *I^q*, *D^q*), B10.T(6R)(*H-2^q*: *K^q*, *I^q*, *D^q*), B10.AKM(*H-2^m*: *K^k*, *I^k*, *D^q*) and B10.BR(*H-2^k*: *K^k*, *I^k*, *D^k*)

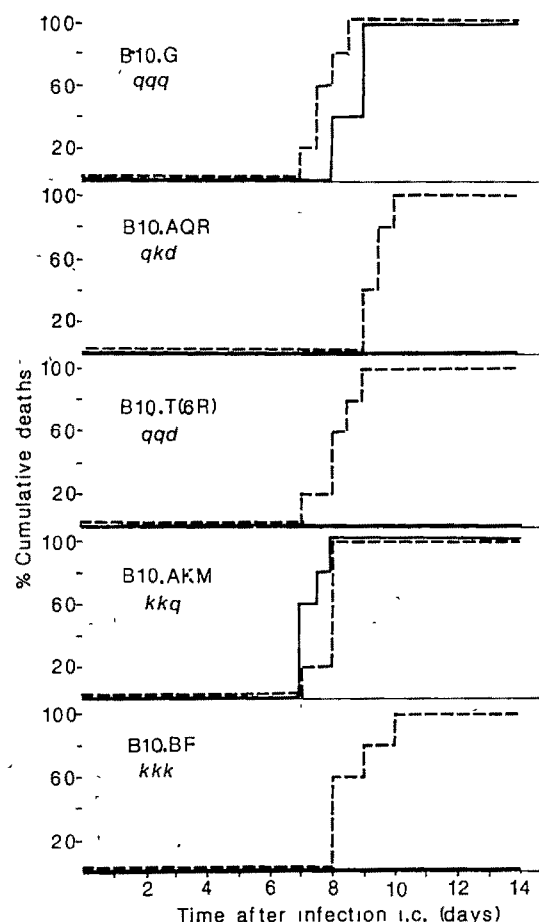


Fig. 1 Groups of five mice of various *H-2* congenic B10 murine strains were injected with ~500 PFU of docile (—) or aggressive (---) UBC-LCMV i.c. and cumulative mortality was monitored. The results shown were obtained with mice purchased from OLAC, Bicester, UK, but identical results were found with mice from the Tierspital, University of Zurich, and from Jackson Laboratory, USA. Mice were between 6 and 18 weeks old. The alleles of the *H-2K*, *I* and *D* regions are shown beneath the strain names

Table 1 LCM mortality of various mouse strains after infection i.c. with 5×10^2 PFU docile or aggressive UBC-LCMV, assessed between day 6 and day 14

Mouse strain	<i>H-2</i>	Non- <i>H-2</i> background	% Mortality due to LCM caused by LCMV	
			Aggressive	Docile
B10.G*†	<i>q</i>	B10	100	100
B10.AKM*†	<i>m</i>	B10	100	100
B10.BR*†‡	<i>k</i>	B10	100	0
(B10.BR × B10.G) _{F1} ‡	<i>k, q</i>	B10	100	100
B10.D2‡	<i>d</i>	B10	100	0
DBA/1*†	<i>q</i>	DBA	100	100
DBA/2*†‡§	<i>d</i>	DBA	0-20	0-20
BALB/c‡	<i>d</i>	BALB	100	0
C3H.Q	<i>q</i>	C3H	20-80	60-100
C3H/HeJ‡	<i>k</i>	C3H	20-80	60-100
CBA/J‡	<i>k</i>	CBA	0-20	60-100
SWR/J†¶	<i>q</i>	Swiss	100	100
NMRI§#	<i>q</i>	Swiss	100	100

Mice (8-16 weeks old) were purchased from or given by: *OLAC, Bicester, UK; †Jackson Laboratory, Bar Harbor, USA; ‡Tierspital, University of Zurich; §Tierversuchsinstitut Hannover, FRG; ||Institut für biologisch-medizinische Forschung AG, 4414 Füllinsdorf, Switzerland; ¶Bantin-Kingman, Aldbrough, Hull, UK; #Savo-Ivanovas, Kisllegg, FRG. The mice were infected i.c. with 30 µl of LCMV Control mice injected with medium alone did not die.

were injected i.c. with ~500 plaque-forming units (PFU) of docile UBC-LCMV. B10.G(*H-2^q*) and B10.AKM(*H-2^m*) mice died with typical LCM whereas B10.BR, B10.AQR and B10.T(6R) mice survived. As B10.AKM(*D^q*) differs from B10.BR(*D^k*) in the *D* region of *H-2* but has in common *H-2K^k*, *I^k* and the B10 background, susceptibility to LCM is linked to *H-2D^q*. The difference in susceptibility of B10.G or B10.AKM compared with B10.BR was seen over a wide range of docile-LCMV doses between 5×10^4 PFU (the highest dose tested) and ~50 PFU i.c. (results not shown). In contrast, aggressive UBC-LCMV injected i.c. over the same dose range (results not shown, except for 500 PFU, in Fig. 1) caused death in all of the above congenic B10 mice irrespective of their MHC.

As LCMV is a T-cell-mediated immunopathological disease, the question arises of whether greater susceptibility reflects greater LCMV-specific cytotoxic T-cell activity. T-cell-mediated immune reactivity was monitored on days 5, 6 and 7 after i.c. infection with docile UBC-LCMV (Table 2, Fig. 2). We found that inflammatory cells in cerebrospinal fluid (CSF) and meninges of susceptible mice were increased from day 5 onwards, yielding values that were 5-10 times greater than those in resistant B10.BR mice on days 6 and 7. Cytotoxic activity was detectable in meningeal infiltrates and spleens of B10.G or B10.AKM mice on day 5 or 6, earlier than the barely detectable activity of B10.BR mice (day 7). The activities measured were much higher in meningeal or spleen cells from susceptible B10.G or B10.AKM than from resistant B10.BR mice (Fig. 2, Table 2).

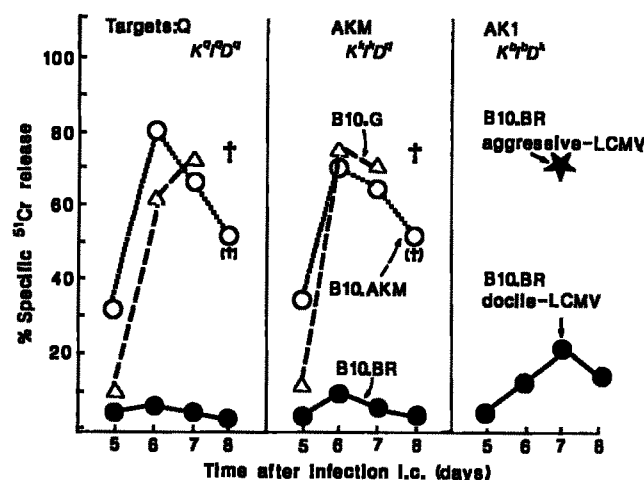


Fig. 2 Two to four B10.G(K^q, I^q, D^q) (Δ), B10.AKM(K^k, I^k, D^k) ($\circ$) and B10.BR(K^k, I^k, D^k) ($\bullet$) mice were injected i.c. with ~ 500 PFU of docile UBC-LCMV on days 5, 6, 7 or 8 before they were killed; all B10.G mice and one of two of the B10.AKM mice had died by day 8 ($\dagger$). The target cells used in the cytotoxicity assay were simian virus 40-transformed fibroblast lines (AKM and AK1) or a methylcholanthrene-induced tumour cell line (Q); these cells had been infected with docile UBC-LCMV for 2 days at a multiplicity of infection of ~ 0.01 . The ^{51}Cr -release assay was run for 3.5–5 h in round-bottomed 96-well plates using 10^4 target cells; effector spleen cells were tested at various effector-to-target ratios (50, 17, 6 and 2:1) (only the results for 50:1 are shown). B10.BR spleen cells taken 7 days after immunization i.c. with aggressive UBC-LCMV were also tested on infected AK1 cells ($\star$). Immune B10.G or B10.AKM spleen cells did not induce release of ^{51}Cr from infected AK1 or L929 cells (data not shown). All effector cells were tested against YAC-1 cells, but no release greater than 7% was measured. Spontaneous release was 20–32%. $\dagger$ Indicates that all or some mice had died.

Most of the cytotoxic activity was found to be $H-2D$ -restricted. B10.G effector cells lysed infected K^q, I^q and D^q targets as efficiently as K^k, I^k and D^k targets, and B10.AKM T cells lysed D^q -compatible infected Q($H-2^q$) targets but either did not lyse or only poorly lysed K^k -compatible infected FS-9($H-2^k$) target cells. For example B10.AKM(K^k, D^k) spleens contained $2\text{--}4 \times 10^2$ lytic units (LU) when measured on infected $H-2K^k$, compared with 18 to 55×10^2 on infected D^q targets

(Table 2); this maps most of the $H-2$ -restricted cytotoxic activity to D^q , in agreement with the finding that most LCMV-specific cytotoxic T-cell activity is usually restricted to D (reviewed in ref. 6). Docile UBC-LCMV immune lymphocytes from B10.BR($H-2^k$) mice lysed infected AKM(K^k, D^k) or AK1(K^k, D^k) targets only to a small extent on days 6, 7 or 8. In contrast, those B10.BR mice that were infected with aggressive UBC-LCMV and usually died of LCMV generated high cytotoxic T-cell activity in meninges and spleen when tested on day 7 (Fig. 2, Table 2), most activity being restricted to D^k (Fig. 2)⁶. Therefore, early and high LCMV-specific MHC class I-restricted cytotoxic T-cell activity correlated positively with susceptibility to LCMV and both T-cell activity and susceptibility to LCMV map to $H-2D$. To our knowledge this represents the first model infection in which susceptibility can not only be shown to depend on the virus strain and on the various host genes but also to be directly regulated by classical transplantation antigens.

The clear correlation between early and high cytotoxic T-cell activity in B10.BR mice infected with aggressive as opposed to docile LCMV was not observed by Pfau *et al.* in 3–4 week-old C3H/HeJ mice tested on day 8 on infected L929 target cells¹³; this discrepancy probably represents an exception and might have been caused by the particular conditions used, that is, young age of the host and only one time point tested on L929 target cells. We found a clear correlation in 8–12-week-old C57BL/6, DBA/1, DBA/2, BALB.B and Swiss mice (R.M.Z., unpublished).

The $H-2$ -dependence of susceptibility to LCMV has been studied by Oldstone *et al.*^{14,15}, who found that, on average, SWR/J($H-2^q$) mice died of LCMV to a greater extent with $\sim 10\text{--}100$ -fold smaller i.c. doses of an aggressive, neurotropic LCMV (strain CA1371) than C3H/HeJ ($H-2^k$) mice. This difference in susceptibility was seen during early and shorter (days 6–10) but not longer (days 6–14) observation periods of the disease, since by day 14 there was no longer a significant difference in susceptibility¹⁴. No clear-cut linkage between susceptibility to LCMV and $H-2$ has been shown since^{15,16}.

Murine LCMV represents a laboratory model in which immunocompetent mice die when infected i.c. but do not usually die if infected intravenously (i.v.) with most LCMV strains^{3–6}. The LCMV model thus demonstrates dramatically that T-cell-mediated disease is observed only if a substantial part of crucial cellular compartments, for example, leptomeningeal cells, are infected and damaged^{3–6,17,18}. Preferential infection by LCMV of leptomeninges, as opposed to other tissues or organs, enhances the lethal outcome of LCMV. If frequencies of precursor

Table 2 LCMV-specific cytotoxic T-cell activity and inflammatory cells in meningeal infiltrates and spleens of mice infected i.c.

Mouse strain (<i>H-2KID</i>)	UBC-LCMV	Meningeal infiltrate							Spleen			LCMV targets used for LU determination (<i>H-2KID</i>)
		LU per brain			Lymphocyte counts ($\times 10^{-4}$)				LU ($\times 10^{-2}$) per spleen			
					per μ l CSF							
						Total cells						
		Day 5	6	7	6	7	6	7	5	6	7	
B10.BR (<i>kkk</i>)	Aggressive	NT	NT	72	NT	4	NT	80	NT	NT	32	FS-9(<i>kkk</i>)
	Docile	<5	<5	10	0.1	0.3	12	27	<1	4	2	FS-9(<i>kkk</i>)
B10.AKM (<i>kkq</i>)	Docile	<5	20	91	0.7	3.5	56	130	4	55	18	Q(<i>qqq</i>)
									<1	4	2	FS-9(<i>kkk</i>)
B10.G (<i>qqq</i>)	Docile	<5	15	160	0.4	4.2	64	160	2	40	28	Q(<i>qqq</i>)

Mice were infected with 5×10^2 PFU of aggressive or docile UBC-LCMV. On the days indicated, mice under ether anaesthesia were bled and their cerebrospinal fluid was tapped as described by Carp *et al.*²⁷; about 5 μl CSF was obtained, diluted 1:10 and counted. The skull was opened, the brain removed and both the skull and brain were rinsed with medium in a Petri dish (total cells). Single meningeal infiltrate cells and spleen-cell suspensions were prepared and assayed at ratios of 50, 17, 6 and 2:1 in a 3 $\frac{1}{2}$ -h test on the following ^{51}Cr -labelled docile-infected target cells: FS-9 ($H-2^k$, given by Dr H. Binz), spontaneous release 18%; and Q ($H-2^q$), 21% spontaneous release. Lytic units (LU), the number of cells giving a 33% specific ^{51}Cr -release by B10.BR effector cells on infected $H-2^k$ target cells and by B10.AKM and B10.G on $H-2^q$ target cells. LU of B10.AKM were also determined on infected FS-9 ($H-2^k$) targets. Total spleen cells varied from 5 to 10×10^7 per spleen. The results shown are for one of two similar experiments using two mice per time point. The data represent the mean values for the two mice. NT, not tested.

effector T cells are high, as is probably the case in immunologically high responders (D^q), their protective activity may limit early on the spread of LCMV to sites other than leptomeninges and therefore increase susceptibility to LCM. The differences in susceptibilities to LCM between docile and aggressive LCMV may also reflect $H-2D$ -regulated differences in cytotoxic T-cell responsiveness that correlate with possible but as yet unrecognized antigenic variations between the two substrains. In fact, Ahmed *et al.* have shown that LCMV-specific T-cell clones may distinguish between various LCMV strains that are serologically indistinguishable¹⁹.

Although the linkage of disease to the MHC has long been recognized²⁰⁻²⁵, despite much speculation, it has remained unclear how MHC-restricted T-cell recognition and MHC-encoded *I*_E gene regulation determine the MHC/disease association^{1,6,22,23}.

The observation, reported here, of a correlation between $H-2D$ -regulated T-cell activity and susceptibility to LCM suggests that many such diseases are the result of a T-cell-mediated pathophysiological mechanism⁹. In general, acute infectious agents probably do not cause disease exhibiting MHC/disease associations because such acute agents select evolutionarily for immunologically high responders in most if not all members of a species. Rather, diseases revealing MHC-linked susceptibility are usually chronic diseases of autoimmune^{24,25}, immunopathological^{6,9} or cancerous nature^{20,21}. The linkage of LCM susceptibility to a major transplantation antigen agrees with the notion⁹ that the association of disease susceptibility with the MHC is often found in connection with non- or poorly cytopathogenic infectious agents that may cause chronic infections without interfering with reproduction. These agents leave a certain amount of leeway for a balance between immune protection

and damage caused by the immune response. Accordingly, MHC-associated disease susceptibility may often signal immunological T-cell-mediated pathogenesis.

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Note added in proof: Our results are compatible with recent studies by Allan and Doherty²⁶ with BALB/c and $C-H-2^{dm2}$ mutant mice. Mutant mice expressing D^{dm2} were less susceptible to the LCMV-Armstrong isolate than BALB/c mice expressing D^d .

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Amplification of P-glycoprotein genes in multidrug-resistant mammalian cell lines

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The multidrug-resistance phenotype expressed in mammalian cell lines is complex. Cells selected with a single agent can acquire cross-resistance to a remarkably wide range of compounds which have no obvious structural or functional similarities¹⁻⁶. The basis for cross-resistance seems to be a decreased net cellular accumulation of the drugs involved, and has been attributed to alterations in the plasma membrane. An over-expressed plasma membrane glycoprotein of relative molecular mass (M_r) 170,000 (P-glycoprotein) is consistently found in different multidrug-resistant human and animal cell lines, and in transplantable tumours^{1,2,6-10}. Consequently, it has been postulated that P-glycoprotein directly or indirectly mediates multidrug resistance⁷⁻¹¹. Here we report the cloning of a complementary DNA encoding P-glycoprotein. Southern blot analysis of hamster, mouse and human DNA using this cDNA as a probe showed that P-glycoprotein is conserved and is probably encoded by a gene family, and that members of this putative family are amplified in multidrug-resistant cells.

A cDNA library was constructed using the expression vector λ gt11 (ref. 12) and messenger RNA isolated from the highly multidrug-resistant cell line, CH^RB30 (see accompanying article)⁶. Plaques were selected by probing with a P-glycoprotein-specific monoclonal antibody, C219 (ref. 6). One

of the presumptive P-glycoprotein cDNA clones, containing an insert of ~600 base pairs (bp), was selected for further study. This cDNA, called λ CHP1 when in the λ gt11 vector, or pCHP1 when subcloned into the pUC9 vector¹³, was transcribed into a 140,000- M_r *lacZ* fusion protein in λ CHP1-infected bacterial cells (Fig. 1A). The size of this fusion protein relative to the uninterrupted β -galactosidase suggested that most of the λ CHP1 insert contained P-glycoprotein coding sequence. Strong evidence that the cDNA insert coded for P-glycoprotein sequences is provided in Fig. 1B, where the same *lacZ* fusion protein was recognized by monoclonal antibodies that identify three independent epitopes of P-glycoprotein⁶.

Further corroboration that the cloned cDNA fragment encodes P-glycoprotein was provided by Northern blot analysis of the highly drug-resistant cell line CH^RB30 and of its sensitive parental cell line, AuxB1 (Fig. 2A). The pCHP1 insert hybridized to a single mRNA component of ~4.7 kilobases (kb), which was strongly expressed in CH^RB30. Slot-blots of mRNA isolated from a series of increasingly multidrug-resistant Chinese hamster ovary (CHO) cells, and from a drug-sensitive revertant cell line (Fig. 2B) revealed hybridization signals consistent with the levels expected if pCHP1 sequences encode a portion of P-glycoprotein. Both the correlation of mRNA expression with multidrug resistance and the size of this mRNA species were consistent with that expected for P-glycoprotein².

Southern blot analysis of multidrug-resistant hamster, mouse and human cell lines and of their drug-sensitive parental cell lines (Fig. 3) demonstrated four main points: (1) in all cases, amplification of pCHP1-homologous sequences correlated with expression of multidrug resistance. The degree of amplification increased with increasing drug resistance in the CHO series, and was lost in the CHO revertant, I10. Similar amplification of pCHP1-homologous sequences has also been detected in a daunorubicin-resistant CHO cell line⁷ (data not shown), and in the adriamycin-resistant Chinese hamster lung cell line LZ¹⁴ (J. H. Gerlach and V.L., unpublished observations). Roninson *et al.*¹⁴ have previously observed 55 kilobase pairs (kbp) of amplified DNA fragments common to both LZ and CH^RC5

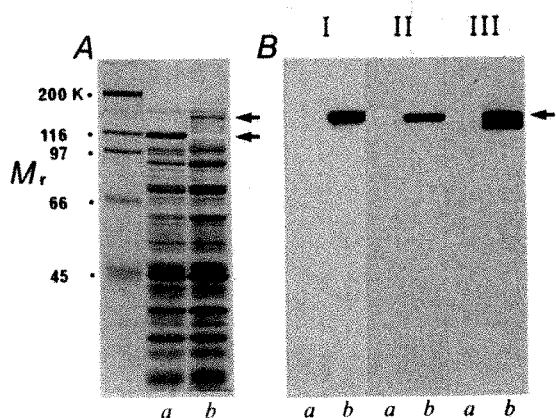


Fig. 1 β -Galactosidase fusion protein containing a P-glycoprotein-specific cDNA insert. **A**, SDS-polyacrylamide gel electrophoresis (PAGE) of aliquots containing 75 μ g protein from lysates of Y1089 bacteria infected with λ gt11 (*a*) or λ CHP1 (*b*). M_r markers in the extreme left-hand lane were as described previously⁹. Arrows indicate the *lacZ* fusion product of λ CHP1 (top arrow) and uninterrupted β -galactosidase (bottom arrow). **B**, Western blots of lanes shown in **A**. Monoclonal antibodies⁶ recognizing three different epitopes on the P-glycoprotein molecule were used. Three identical blots were probed with representative monoclonal antibodies of group I (C219 probe), group II (C32 probe) and group III (C494 probe), as indicated.

Methods. The cDNA probe, λ CHP1, was obtained as follows. Poly(A)⁺ RNA was isolated from the highly colchicine-resistant cell line, CH^RB30, by standard techniques^{22,23}. cDNA was synthesized and cloned into the β -galactosidase-encoding *lacZ* gene of the bacteriophage λ gt11 (ref. 12) by the methods of Huynh *et al.*²⁴. The library contained 1.3×10^6 recombinants, 65% of which contained cDNA inserts. Screening was done with radioiodinated ($\sim 10 \mu\text{Ci } \mu\text{g}^{-1}$) monoclonal antibody C219. After plaque purification, the insert (~ 600 base pairs, bp) from one of the positive clones, λ CHP1, was subcloned in the plasmid pUC9 (ref. 13). This recombinant, designated pCHP1, was used in subsequent hybridization analyses. Infection of Y1089 bacteria with either λ gt11 or λ CHP1 and induction of the *lacZ* products with isopropyl β -D-thiogalactopyranoside were done as described¹². Bacteria were lysed by brief sonication, solubilized and then subjected to SDS-PAGE according to Laemmli²⁵. Western blot analyses were done according to Towbin *et al.*²⁶, as described previously⁹.

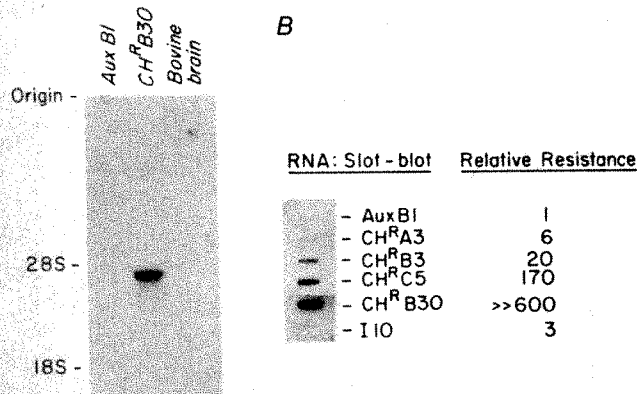


Fig. 2 RNA analysis using the pCHP1 probe. **A**, Northern blots of total cellular RNA from drug-sensitive AuxB1 and highly multidrug-resistant CH^RB30 cells. The third lane contained RNA from normal bovine brain as a control. **B**, Slot-blot analysis of RNA from a series of CHO cells with different levels of drug resistance. The relative resistance to colchicine is indicated opposite each slot^{1,2}. The clonal lines CH^RA3, CH^RB3 and CH^RC5 were derived from sequential selections for increasing colchicine resistance; CH^RB30 was obtained by continuous culture of CH^RC5 in increasing colchicine concentrations from 5 to 30 $\mu\text{g ml}^{-1}$, and revertant I10 was selected from CH^RC5 in a single step^{2,6}. The level of P-glycoprotein expression has been shown to correlate with the degree of drug resistance^{6,9}. Northern and slot blots were performed as described by Thomas²⁷. The probe used was pCHP1 insert, nick-translated to a specific activity of $\sim 10^8$ d.p.m. μg^{-1} .

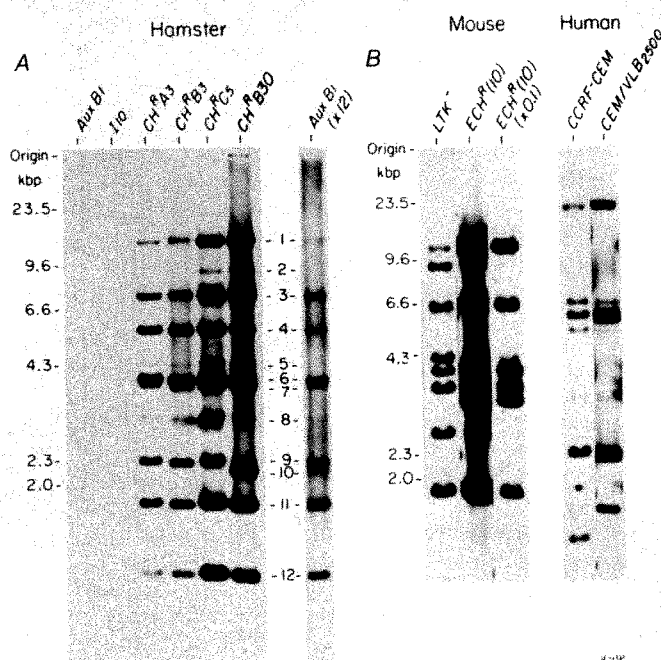


Fig. 3 DNA analysis with the pCHP1 probe. Southern blots of *Eco*RI-digested genomic DNA from: **A**, the series of increasingly multidrug-resistant CHO cells shown in Fig. 2; and **B**, multidrug-resistant mouse and human cells. Multiple bands were also observed when other restriction enzymes were used with CHO DNA (not shown). Note the appearance of new bands, probably 'novel joints'¹⁵, not detected in sensitive parental cells (for example, band 2 in CH^RC5 and CH^RB30). Quantitative densitometry of the CH^RB30 band pattern in a separate experiment revealed that restriction fragments 4, 6 and 9 were amplified 10–20-fold, while fragments 7, 10, 11 and 12 were amplified 50-fold or more.

Methods. DNA was digested with *Eco*RI and electrophoresed in 0.6% agarose gels. Transfer to nitrocellulose was performed essentially as described by Southern²⁸. Hybridization was in 50% formamide, 5 $\times$ SSC, 20 mM sodium phosphate pH 6.5, 200 $\mu\text{g ml}^{-1}$ sonicated salmon sperm DNA, 0.1% bovine serum albumin (BSA), 0.1% Ficoll, 0.1% polyvinylpyrrolidone, 10% dextran sulphate, plus 10^6 d.p.m. ml^{-1} pCHP1 insert nick-translated to a specific activity of $\sim 10^8$ d.p.m. μg^{-1} . Filters were washed twice for 5 min each in 2 $\times$ SSC, 0.1% SDS at room temperature, twice for 30 min in the same solution plus 0.1% sodium pyrophosphate at 50 $^{\circ}\text{C}$, and twice for 30 min in 0.1 $\times$ SSC, 0.1% SDS and 0.1% sodium pyrophosphate at 50 $^{\circ}\text{C}$. M_r s were determined from *Hind*III-digested λ DNA, which was co-electrophoresed. Hamster cell lines were those described in Fig. 2 legend. The resistant mouse cell line was derived from L cells and was selected with colchicine¹⁰. CEM/VLB₂₅₀₀ is a highly resistant human leukaemic cell line selected for growth in 2,500 ng ml^{-1} vinblastine²⁰, from the resistant subline CEM/VLB₁₀₀ (ref. 4). CCRF-CEM is the drug-sensitive parental cell line. Autoradiographs of hamster DNA were for 15 h, except for the lane marked AuxB1 ($\times 12$), which was exposed 12 times longer. Autoradiographs of mouse and human DNA were for 7 days, except the lane marked ECH^R(10) ($\times 0.1$), which was exposed for one-tenth of that time. Band 1 in AuxB1 is under-represented in this experiment.

using a DNA renaturation technique in agarose gels; in view of our present findings, the P-glycoprotein genes probably comprise a major portion of those sequences.

(2) The pCHP1 insert detected multiple bands in the Southern blots of *Eco*RI-digested DNA, despite the fact that this cDNA fragment was only ~ 600 bp long and contained no internal *Eco*RI sites. Although other explanations are possible, the simplest is that P-glycoprotein is encoded by a multigene family. In support of this notion, only a subset of the 10 bands detected in CHO cells were transfected (and these were amplified) in mouse cells transformed to the multidrug-resistant phenotype with DNA derived from multidrug-resistant hamster cells R.-p. Du, K.D. and V.L., unpublished observations).

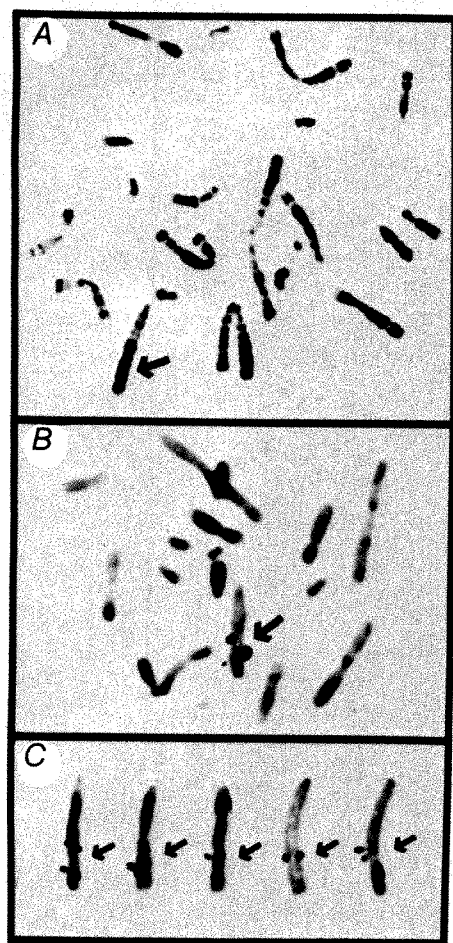


Fig. 4 Cytological evidence for localization of amplified P-glycoprotein sequences to a homogeneously staining region (HSR) on the Z4 chromosome of $CH^R B30$. **A**, Representative G-banded $CH^R B30$ cell demonstrating an HSR on chromosome Z4 (arrow). The most consistent and characteristic chromosomal alteration between the parental line AuxB1 and the highly colchicine-resistant $CH^R B30$ line was the presence of an HSR on the long arm of chromosome Z4. **B**, Localization of autoradiographic grains to the Z4 HSR (arrow) following hybridization *in situ* with ^3H -labelled pCHP1. **C**, Examples of grain localization to the Z4 HSR (arrows) from five different $CH^R B30$ cells as in **B**. Of 25 cells examined, 20 yielded one or more autoradiographic grains localized to the Z4 HSR, and 45% (60/133) of all grains were localized to the Z4 HSR. No significant grain localization in the drug-sensitive cell AuxB1 was observed in the same conditions (data not shown). **Methods.** G-banding and *in situ* hybridization analysis were performed as described previously^{29,30}. Cells were hybridized for 15.5 h at a final DNA concentration of $0.65 \mu g ml^{-1}$, followed by exposure for 7 days before autoradiographic development (**B**, **C**). The autoradiographic analysis was performed with unbanded chromosomes; however, the size and arm length ratio of the Z4 HSR allowed it to be unequivocally identified in $CH^R B30$ cells.

(3) The Southern blots also indicate that the presumptive CHO P-glycoprotein gene family appears to be clustered within one amplification unit (an amplicon), because in $CH^R A3$, which was selected in a single step from the sensitive parent AuxB1, all the restriction fragments were simultaneously amplified by ~10–20-fold. Coordinate amplification did not occur in mouse and human cell lines, suggesting that the P-glycoprotein genes detected in these cases were not part of the same amplicon. The appearance of amplified fragments that have no counterparts in parental DNA may represent novel joints. Such regions are found in tandemly amplified sequences¹⁵.

(4) The ability of the pCHP1 insert to detect human sequences under stringent conditions provided evidence that P-glycoprotein is conserved at the DNA level, as well as at the protein level⁶.

Karyotypic markers of gene amplification—homogeneously staining regions or double minute chromosomes—have been described in multidrug-resistant cell lines^{3,11,16–19}. In the CHO cell system, there exists an extensive homogeneously staining region on the Z4 chromosome in $CH^R B30$ cells (Fig. 4) that is not found in the drug-sensitive parental cell line. *In situ* hybridization using ^3H -labelled pCHP1 revealed that the amplified P-glycoprotein sequences reside within the Z4 homogeneously staining region. No significant grain localization to other chromosomes was apparent.

The fact that multidrug resistance is associated with gene amplification suggests that the basis of this phenotype is an increased expression of a normal gene product. It may be speculated that the function of P-glycoprotein in normal cells is to modulate cellular permeability to diverse compounds and that overexpression of P-glycoprotein in multidrug-resistant cells results in an exaggeration of this normal function. The present study raises the possibility that different members of the putative P-glycoprotein gene family control different aspects of permeability, and that the sum of their individual effects produces the diversity of the multidrug-resistance phenotype. Differential expression of members of this family may also result in the variability in resistance patterns among different multidrug-resistant cell lines. The cloning and transfection of a functional P-glycoprotein gene will provide insight into these hypotheses and will determine whether or not overexpression of P-glycoprotein alone is sufficient to mediate the multidrug-resistance phenotype.

Recently, we have detected P-glycoprotein overexpression in some cases of advanced ovarian cancer²⁰. This suggests that the multidrug-resistance mutation can occur in human malignancies and result in non-responsiveness of the malignancy to combination chemotherapy^{6,20,21}. Further studies are required to determine the generality of this finding among other malignancies. The homology of pCHP1 to human P-glycoprotein sequences should facilitate studies in this area.

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Detection of P-glycoprotein in multidrug-resistant cell lines by monoclonal antibodies

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One reason for the failure of chemotherapy in the treatment of advanced cancers may be the outgrowth of multidrug-resistant tumour cells¹⁻⁵. Multidrug resistance has been modelled in numerous mammalian cell lines in which the phenotype is characterized by a pleiotropic cross-resistance to unrelated drugs¹⁻⁷. In the study reported here, we have produced monoclonal antibodies whose binding to plasma membranes of different multidrug-resistant mammalian cells correlates with the degree of drug resistance. All these antibodies are specific for P-glycoprotein, a cell surface component of relative molecular mass (M_r) 170,000 (170K) that has been described previously⁸⁻¹⁵, and are directed against three spatially distinct epitopes which define a conserved cytoplasmic domain in the C-terminal region of the P-glycoprotein polypeptide. The conserved nature of P-glycoprotein and its low-level expression in drug-sensitive cells suggest that it has an important function at the cell surface. The monoclonal antibodies against P-glycoprotein described here might serve as diagnostic reagents for clinically unresponsive tumours¹⁶.

Hybridomas were produced from splenocytes of mice immunized with SDS-solubilized plasma membranes of multidrug-resistant Chinese hamster ovary (CHO) and human cell lines. Primary screening of the hybridomas was achieved using nitrocellulose test strips spotted with SDS-solubilized plasma membranes of drug-sensitive and multidrug-resistant CHO and human cells (for details see Fig. 1 legend). By this means we were able to select hybridomas secreting monoclonal antibodies specific for drug resistance-associated antigens in either CHO or human cells. Screening with denatured antigens immobilized on nitrocellulose ensured that the monoclonal antibodies selected would be of use in the detection of antigens in protein immunoblots (Western blots). Eight stable hybridoma clones were ultimately isolated, yielding three monoclonal antibodies which appeared to be specific for resistance-associated CHO antigens, and five that bound antigens common to multidrug-resistant CHO and human plasma membranes.

To characterize the above differences in binding specificity more precisely, supernatants from cloned hybridomas were screened with a larger panel of plasma membranes from drug-sensitive and multidrug-resistant cell lines (Table 1). The results (Fig. 1) showed that the monoclonal antibodies could be classified into three distinct groups according to the pattern of staining obtained in the dot-blot panel. No significant staining of drug-sensitive cell membranes was observed with any of the eight monoclonal antibodies. The antibodies in group I stained membranes of all drug-resistant cells tested, while those of group II failed to stain drug-resistant human membranes. The single member of group III stained drug-resistant CHO and human membranes, but not those of mouse. Further evidence of distinct epitopes was provided by competitive binding assays, as shown in Fig. 2. The latter results clearly allowed the classification of the monoclonal antibodies into three groups (I, II and III) on the basis of their binding to three spatially distinct epitopes.

The antigen specificity of each of the monoclonal antibodies was determined by probing plasma membrane components in Western blots. Figure 3A shows the results for plasma membranes from various cell lines (Table 1). Bands of $M_r \sim 170K$ were stained in lanes representing CHO cells of increasing colchicine resistance (Fig. 3A, lanes b-d), and a daunorubicin-resistant CHO cell line (lane e). Similar results were obtained

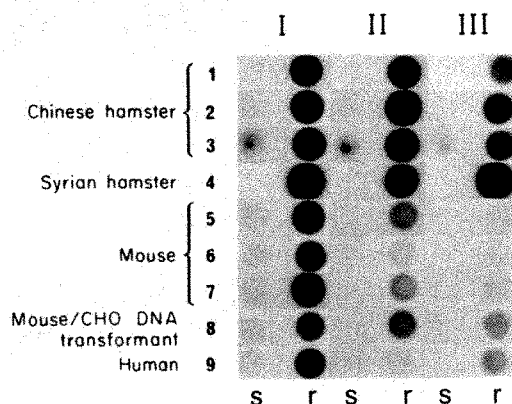


Fig. 1 Selection and characterization of monoclonal antibodies, using immunoblotting techniques. Dot immunoblots were used in a primary screening assay to select hybridomas of interest (see below). Shown here are dot immunoblots which were used in a more comprehensive secondary screening of re-cloned hybrids. Eight hybridomas were tested against a panel of SDS-solubilized plasma membranes purified from pairs of matched drug-sensitive (s) and drug-resistant (r) cells; 2 μ l (2 μ g protein) of each were spotted onto nitrocellulose filters. Hybridoma supernatants were adjusted to a monoclonal antibody concentration of 1 μ g ml⁻¹. Note the differential staining of drug-resistant cell membranes. Three different staining patterns were observed and the eight monoclonal antibodies were classified accordingly into groups I, II and III. Comparison of rows 4, 5 and 9 emphasizes major differences in specificity between the groups. The assignment of monoclonal antibodies to groups was as follows. Group I: C11, C26, C36, C219; group II: C32, C103, C699; group III, C494. Their isotypes were κ 2a, except for C11, C26 and C36, which were κ 1. Test cell lines were (sensitive line, resistant line): (1) AuxB1, CH^RB30; (2) AuxE29, DNR^R159; (3) CHEF 18, VCR 1.5; (4) C12TSV₂S, C12TSV₂R₂; (5) LMTK^R, ECH^R; (6) S180, S180A5; (7) UV-2237, UV-2237-ADM^R; (8) LTA, LC5B1; (9) CCRF-CEM, CEM/VLB₅₀₀ (see Table 1 for description of cell lines).

Methods. Twelve-week-old (BALB/c $\times$ 3H)F₁ mice were immunized with injections of purified plasma membranes isolated according to Riordan and Ling¹⁰ from CH^RB30 and CEM/VLB₅₀₀ cell lines. Membrane protein was assayed according to a modification¹² of the method of Lowry *et al.*³³. Membranes were solubilized in a 2% SDS-containing buffer as for SDS-PAGE^{11,12}, with a protein/SDS ratio of 1:1.4, and were emulsified 1:1 with Freund's incomplete adjuvant. The first injection consisted of 200 μ g of CH^RB30 membrane given intraperitoneally, followed after 14 days by a second, identical injection. After 14 months the mice were injected with a mixture of 100 μ g each of CH^RB30 and CEM/VLB₅₀₀ plasma membranes. Identical injections were given 14 and 21 days after the first injection in this series. In addition, on day 21 a mixture containing 100 μ g of sonicated plasma membrane vesicles from each of CH^RB30 and CEM/VLB₅₀₀ was injected intravenously. On day 26, spleens were removed aseptically and splenocytes fused 3:1 with the Sp2/0-Ag14 fusion partner^{34,35}. Cells were plated in 96-well plates at 10⁵ myeloma cells per well in RPMI-1640 medium containing 100 μ M hypoxanthine, 0.5 μ M aminopterin, 30 μ M thymidine, 2 mM L-glutamine, 50 μ M 2-mercaptoethanol and 15% fetal bovine serum. When wells seemed to be 25% confluent (50–80% of wells after 10–14 days), they were screened for specific antibody-producing hybrids. Primary screening of individual hybridoma supernatants was done using a panel of SDS-solubilized plasma membranes, by dot blotting. Test strips were prepared by spotting 1 μ l of SDS-solubilized plasma membrane (1 mg ml⁻¹ protein; prepared as for SDS-PAGE^{11,12}) onto numbered 5 $\times$ 25 mm nitrocellulose strips. Each strip was spotted with plasma membranes from the drug-sensitive CHO cell line AuxB1, the drug-resistant CHO line CH^RB30, the drug-sensitive human cell line CCRF-CEM, and the drug-resistant human line CEM/VLB₅₀₀ (see Table 1 for description of cell lines). The dried dot blots were blocked in 3% bovine serum albumin (BSA)/saline³⁶ for 1 h at 37 $^{\circ}$ C, then placed in test tubes containing hybridoma supernatants diluted 1:10 in BSA/saline. After overnight incubation at 4 $^{\circ}$ C, the strips were washed in several changes of phosphate-buffered saline (PBS), then developed with ¹²⁵I-labelled IgG goat anti-mouse immunoglobulins (Cappel; 10⁶ c.p.m. ml⁻¹ in 3% BSA/saline) for 6 h at 25 $^{\circ}$ C. After washing and drying, the test strips were exposed overnight, with intensifying screens, on Kodak X-AR5 film at -70 $^{\circ}$ C. About 1,000 strips were processed simultaneously. Candidate wells were expanded and cloned twice by limiting dilution. The above screening method was repeated to pick positive clones. Secondary screening, as shown, was done in a similar manner, but using plasma membranes derived from several different cell lines. Eight stable clones were finally isolated.

when blots of colchicine-resistant mouse cells (Fig. 3A, lane h) and vinblastine-resistant human leukaemic cells (lane j) were probed (compare with drug-sensitive parental cells in lanes a, g and i, and a drug-sensitive revertant, lane f). All three classes of monoclonal antibody stained bands of identical M_r when a

side-by-side comparison of Western blots was made (data not shown). Previous work has established that homologous P-glycoproteins with a consistent M_r of 170K are expressed in all the multidrug-resistant cell lines tested here¹¹⁻¹⁵. Longer exposure of the blots (Fig. 3A, lanes k-n) revealed faint bands

in the membranes of drug-sensitive parents and revertant cells in lanes a, f, g and i, respectively. Low-level expression of P-glycoprotein in drug-sensitive cells has been reported previously¹². Trivial explanations for the apparent homologies of the 170K bands in different species, such as detection of pros-

Table 1 Description of cell lines

	Parental line	Resistant line	Drug of selection	Selection conc. ($\mu\text{g ml}^{-1}$)	Relative resistance	Cross-resistance	Ref.
Chinese hamster	AuxB1	CH ^R A3	CCH	0.10	7	PUR ETB DNR CCH ERY	23, 24
		CH ^R B3	CCH	3.0	31	PUR ETB DNR CCH EME ACR	23, 24
		CH ^R C5	CCH	10	145	CYB ERY	23, 24
	AuxE29	CH ^R B30	CCH	30	640	CCH GRD PUR DNR EME VLB DOX	23, 24
		DNR ^R 51	DNR	0.50	41	CMD ACR ETB CYB ERY PRO	*
		DNR ^R 159	DNR	1.0	37	CCH PUR DOX	11
		CP-3.7	PHA	—	—	DNR PUR DOX CCH VLB EME	11
		I10	—	—	—	VLB DNR PUR DOX CCH	14
		CHEF 18	VCR	1.5	2	CCH PUR DNR	25
		Cl ₂ TSV ₅ S	DAC	2.0	40	CCH CMD VLB DNR PUR	26
		LMTK ^R	CCH	0.50	1,750	VCR PUR CMD	27, 28
Syrian hamster	UV-2237	UV-2237-ADM ^R	DOX	1.0	26	CCH PUR CYB DAC EME	15
	S180	S180A5	DOX	~1	140	VCR DNR VLB MMC AMS DAC ADX	13, 29
Mouse/CHO DNA transformant	LTA	LC5B1	CCH	0.50	40	DNR MRM ADX VCR DAC	30
					43	PUR CCH EME CYB DAC	15
Human	CCRF-CEM	CEM/VLB ₁₀₀	VLB	0.10	70	(VCR VLB PUR CCH DNR EME)*	31
		CEM/VLB ₅₀₀	VLB	0.50	~400	NA	*
		CEM/VLB ₁₀₀₀	VLB	1.0	~800	VCR VLB PUR DNR DOX	*
		CEM/VLB ₅₀₀₀	VLB	5.0	~4,000	NA	*

Cell lines of the series CH^RA3, CH^RB3 and CH^RC5 were clonally selected, stepwise, from AuxB1 (ref. 23). CH^RB30 was selected by continuous culture, in increasing colchicine (CCH) concentrations, from CH^RC5. CP-3.7 is a phytohaemagglutinin (PHA)-resistant mutant, selected from CH^RC5, which retains the multidrug resistance phenotype of CH^RC5 (ref. 14). I10 is a revertant cell line, selected in a single step from CH^RC5 (ref. 25). CEM/VLB₁₀₀ was obtained from Dr W. T. Beck³¹ and was cloned in methylcellulose containing 0.1 $\mu\text{g ml}^{-1}$ vinblastine (VLB). The series of VLB-resistant human leukaemic cell lines, CEM/VLB₅₀₀, CEM/VLB₁₀₀₀ and CEM/VLB₅₀₀₀, was derived from a clone of CEM/VLB₁₀₀ by continuous growth in increasing drug concentrations and selection at 0.5, 1.0 and 5.0 $\mu\text{g ml}^{-1}$ vinblastine, respectively. Relative resistance was determined as described previously²⁴. Relative resistance values for the CH^R CHO series and CEM/VLB series cells were re-assayed for the present work and may not agree exactly with previously reported values. Drugs that were tested for cross-resistance are listed in order of decreasing relative cross-resistance. ACR, acriflavine; ADX, AD-32; AMS, amsacrine; CMD, colcemid; CYB, cytochalasin B; DAC, actinomycin D; DNR, daunorubicin; DOX, adriamycin; EME, emetine; ERY, erythromycin; ETB, ethidium bromide; GRD, gramicidin D; MMC, mitomycin C; MRM, marcellomycin; PRO, proflavine; PUR, puromycin; VCR, vincristine. NA, not assayed.

* Present study.

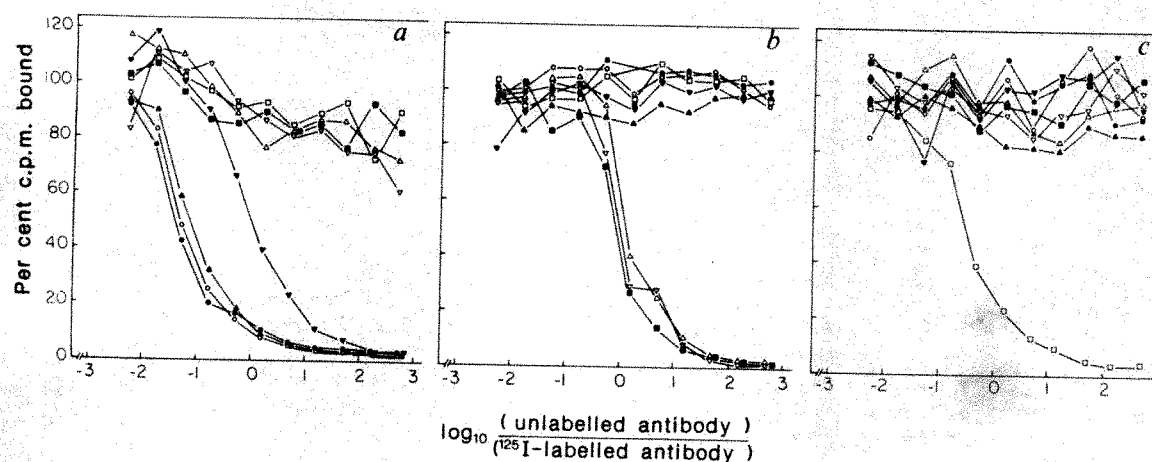
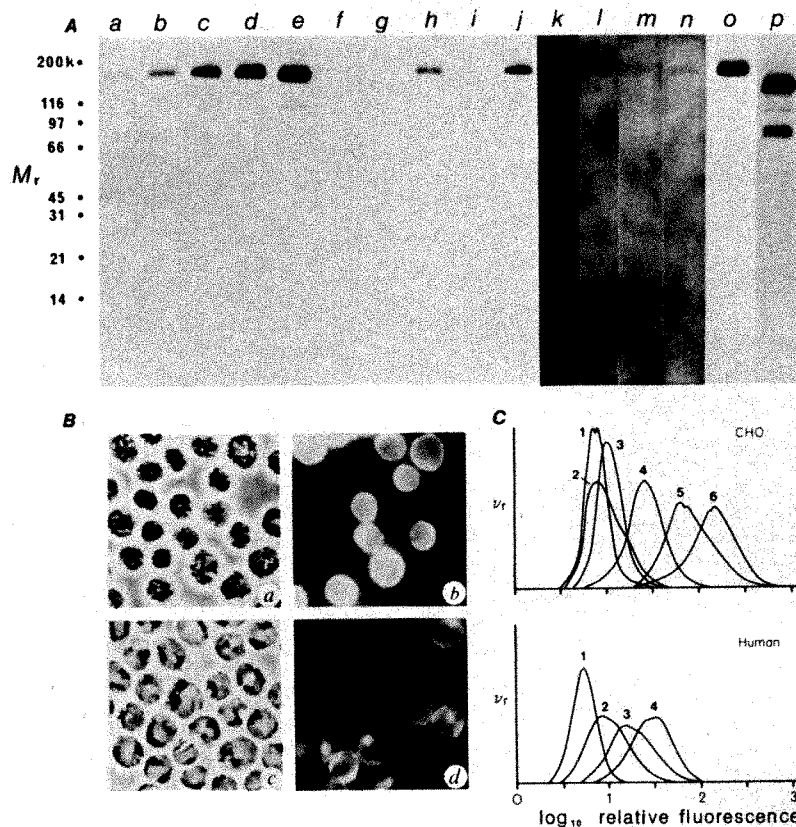


Fig. 2 Direct competitive binding assay of eight monoclonal antibodies against purified, radioiodinated antibody. Target antigen was plasma membrane vesicles from CH^RB30, immobilized on nitrocellulose in 96-well plates. The abscissa is a logarithmic scale of the ratio of the concentrations of unlabelled competing antibody and radiolabelled antibody. The ordinate shows the percentage of c.p.m. bound, normalized to c.p.m. bound in the absence of competing antibody. Results of single simultaneous experiments are shown for clarity. **A**, Competition between a labelled group I monoclonal antibody (C219) and dilutions of eight different ascites fluids. No group II or III antibodies competed with C219. The shift in the competition curves of other group I antibodies with respect to the self-competition curve of C219 probably reflects differences in binding affinity. **B**, Competition between the group II antibody C32 and other ascites fluids. Monoclonal antibodies of groups I and III did not compete with C32. The binding kinetics of the three group II antibodies appeared to be similar. **C**, Competition data for the single representative of group III (C494). No group I or group II antibodies competed with C494. The monoclonal antibodies tested were: ○, C11; ●, C26; △, C32; ▲, C36; ▽, C103; ▼, C219; □, C494; and ■, C699.

Methods. Millititre HA plates (Millipore) were coated with 1 μg of CH^RB30 plasma membrane in 30 μl PBS per well, and incubated overnight at 37 °C. Wells were blocked with 100 μl of 10% BSA in PBS for 4 h at 37 °C. After washing with PBS, the wells were incubated with 30 μl of radioiodinated, purified monoclonal antibody (20 ng, 100,000 c.p.m.) mixed with 30 μl of ascites fluid serially diluted threefold in 10% BSA/PBS. After incubation for 16 h at 25 °C, the plates were washed with PBS containing 0.1% Tween 20. Dried wells were punched out, and bound ¹²⁵I-labelled antibody was quantitated by γ -counting. Monoclonal antibodies were purified by chromatography on protein A-Sepharose (Pharmacia) and concentrated by ammonium sulphate precipitation³⁷. Purified antibodies were radioiodinated using chloramine T³⁸. C219 protein was quantitated gravimetrically ($\pm 5\%$) by lyophilizing the purified antibody and drying to constant weight, *in vacuo* at 25 °C. Other purified monoclonal antibodies were quantitated by absorbance at 280 nm in PBS against C219 standards. Clarified ascites were titred by spotting twofold serial dilutions onto nitrocellulose and probing with radiolabelled goat anti-mouse immunoglobulins. Spots were compared by autoradiography and densitometry, using purified C219 as a standard. Ascites of Sp2/0-Ag14 cells gave no significant background.

Fig. 3 Specific staining of P-glycoprotein in Western blots and in cells. **A**, Western blots of plasma membranes of drug-sensitive and resistant cells (Table 1), using monoclonal antibody. A group I antibody (C219) was used throughout this figure. Lanes *a*–*d*, CHO cells of increasing drug resistance: *a*, AuxB1; *b*, CH^RB3; *c*, CH^RB3; *d*, CH^RC5; *e*, daunorubicin-resistant CHO cell line, DNR^R51; *f*, drug-sensitive revertant of CH^RC5, I10; note that the staining intensity of the 170K P-glycoprotein band correlates with the degree of drug resistance (Table 1); *g*, sensitive and colchicine-resistant mouse cells (LMTK⁺, ECH^R); *i*, *j*, sensitive and vinblastine-resistant human cells (CCRF-CEM, CEM/VLB₁₀₀); *k*–*n*, eightfold longer exposures of lanes *a*, *f*, *g* and *i*, respectively, showing staining of the 170K band in membranes of drug-sensitive cells; lane *o*, same loading as in *d*, but the blot was treated with bacterial alkaline phosphatase (10 U ml⁻¹ in 10 mM Tris-HCl (pH 8.0), 2 h at 25 °C) according to Davis *et al.*²⁹ before probing with monoclonal antibody. Phosphatase treatment did not affect antibody binding (compare with lane *d*). The same result was obtained when the plasma membrane sample was treated with phosphatase before SDS-PAGE. *p*, CP-3.7, showing that depletion of N-linked carbohydrate¹⁴ on P-glycoprotein (note the shift in *M_r*) did not affect antibody binding. Minor bands of lower *M_r* are probably degradative fragments of the major band. SDS-PAGE according to Fairbanks *et al.*⁴⁰ and Western blots according to Towbin *et al.*³⁶ were performed as described previously^{11,12}, using 50 µg of membrane protein per lane. Protein staining of gels (not shown) gives a complex pattern of at least 30 bands^{11,12}. **B**, Indirect immunofluorescence staining of drug-resistant cells with monoclonal antibody. Mixtures of cell were stained with C219 and fluorescent second antibody: *a*, phase-contrast photomicrograph of a 1:1 mixture of AuxB1 and CH^RB30 cells; *b*, fluorescence photomicrograph of same field as in *a*; *c*, phase-contrast photomicrograph of a 1:1 mixture of CCRF-CEM and CEM/VLB₅₀₀₀ cells; *d*, fluorescence photomicrograph of same field as in *c*. AuxB1 or CCRF-CEM cells alone (not shown) showed no significant fluorescent staining in these conditions. Fields of CH^RB30 or CEM/VLB₅₀₀₀ cells alone (not shown) showed similar staining of all cells. **C**, Flow cytometry of indirect immunofluorescence staining using cell lines of different drug resistance. The abscissa is a logarithmic scale of fluorescence intensity relative to calibration beads (see below). The ordinate shows the relative frequency (*v_r*) of cells in a given fluorescence intensity channel. Typically about 10,000 cells were analysed in 256 channels for each curve. The top panel shows a series of CHO cell lines, the bottom panel a series of human cell lines. Peaks are identified as follows. Top panel: 1, AuxB1; 2, I10; 3, CH^RA3; 4, CH^RB3; 5, CH^RC5; 6, CH^RB30. Bottom panel: 1, CCRF-CEM; 2, CEM/VLB₁₀₀; 3, CEM/VLB₅₀₀; 4, CEM/VLB₅₀₀₀. Staining was done with the monoclonal antibody C219, and fluorescein-conjugated second antibody (see below). Note the correlation between the degree of drug resistance (Table 1) and fluorescence staining intensity. **Methods.** For fluorescence microscopy, cells were fixed in 0.46% formaldehyde in PBS (pH 7.0), on ice for 2–3 min, after which they were washed twice with PBS at 4 °C and fixed further in 95% ethanol at –20 °C for 5 min, then in acetone at –20 °C for 5 min. Cells were pelleted at 4 °C, the acetone was aspirated and the pellet air-dried for 5 min before staining. For flow cytometry, cells were fixed only in 70% methanol at –20 °C for 5 min, and washed twice in PBS before staining. Cells (10⁶ ml⁻¹) were stained in 1% normal rabbit serum (NRS)/PBS containing monoclonal antibody (~3 µg ml⁻¹) from diluted ascites for 1 h at 4 °C with inversion. Cells were washed twice with 1% BSA/PBS, then incubated in fluorescein-conjugated rabbit anti-mouse immunoglobulin (Dako) diluted 50-fold in 1% NRS/PBS for 1 h at 4 °C. Cells were washed three times in 1% BSA/PBS and resuspended in 100 µl of 50% glycerol in PBS containing 20 mg ml⁻¹ 1,4-diazabicyclo(2,2,2)octane. Stained cells were viewed and photographed using a Zeiss epifluorescence microscope fitted with an H485 filter pack. For flow cytometry, cells were resuspended to 10⁶ ml⁻¹ in 1% BSA/PBS and analysed in a Coulter EPICS V flow cytometer. The logarithm of integrated fluorescein isothiocyanate fluorescence was determined with forward-angle light scatter gated to analyse only single cells. Approximately 10⁴ cells were analysed per cell line. Fluorescence intensity was standardized against Coulter 10 µm Full Bright calibration beads at 10-fold and 100-fold attenuation.



thetic groups like phosphate, or common carbohydrate epitopes, are excluded by the controls in Fig. 3A, lanes *o* and *p*: here the absence of phosphate or N-linked carbohydrate moieties, respectively, had no apparent effect on binding of antibody to the antigen. Based on the correlation of increased expression of the antigen with increased multidrug resistance, and the conservation of structure of the antigen irrespective of the drug of selection in a number of mammalian species, we concluded that the antigen bound by each of the monoclonal antibodies was P-glycoprotein, as defined previously¹².

In many diagnostic and research applications, it is important for an antibody to be capable of detecting the antigenic site as it is presented in cells and tissues. In this regard we have found, using both radiolabelled monoclonal antibodies and indirect immunofluorescence staining, that none of the monoclonal antibodies binds the surface of intact multidrug-resistant cells (data not shown). Nevertheless, as shown in Fig. 3B, using fixed and permeabilized cells, the monoclonal antibodies labelled the surface membrane of resistant CHO and human cells, demonstrating their potential as reagents for the diagnostic detection of P-glycoprotein in fixed cells and tissues. In Fig. 3C, flow cytometry of cells stained with the monoclonal antibodies showed that the antibody binding correlated with the degree of

drug resistance of the cells, much like the specific P-glycoprotein staining in Fig. 3A. Thus, the staining of permeabilized cells appeared to be due to specific binding of monoclonal antibodies to epitopes of P-glycoprotein exposed on the cytoplasmic aspect of the plasma membrane.

The group I monoclonal antibody C219 has been used to obtain a 600-base pair (bp) cDNA probe, λ CHP1, from a λ gt11 expression library of the multidrug-resistant CHO cell line, CH^RB30 (see accompanying paper¹⁷). The *lacZ* fusion product of λ CHP1, which expresses a C-terminal fragment of CHO P-glycoprotein, is recognized not only by the monoclonal antibody C219, but by all eight antibodies described here¹⁷. Localization of three independent epitopes to a peptide not more than 200 amino acids long was a surprising result as this represents only ~15% of the P-glycoprotein polypeptide. This observation is consistent, however, with the notion that the monoclonal antibodies detect a cytoplasmic domain in fixed cells, as the C-terminus of a transmembrane glycoprotein is often situated on the cytoplasmic side of the membrane. The observation that the monoclonal antibodies bind a fusion protein produced in bacterial cells further confirms that they are directed against the polypeptide moiety of the P-glycoprotein.

The clustering of all three epitopes in the C-terminus of

P-glycoprotein suggests that this region is relatively more immunogenic than most of the protein. The C-terminus possibly is part of a hydrophilic domain in an otherwise relatively hydrophobic protein. Alternatively, it may be a region of the polypeptide that is conformationally more fluid and, therefore, is more likely to be antigenic¹⁸⁻²⁰. It is significant that all eight monoclonal antibodies showed specificity for the same plasma membrane component, supporting the notion that the expression of P-glycoprotein is the major alteration of the multidrug-resistant cell surface. One of the epitopes described here (identified by group I antibodies) seems to be highly conserved among species, but the other two appear to be expressed in a species-dependent manner, as seen in Fig. 1. The latter epitopes may represent more variable regions of P-glycoprotein structure.

The monoclonal antibodies described here will serve as sensitive and specific reagents for the detection of P-glycoprotein in experimental systems and in normal and neoplastic tissues. Furthermore, they have potential for use in screening human tumour biopsies, where early detection of elevated levels of P-glycoprotein might indicate the need for a departure from routine chemotherapy and the application of novel treatments to circumvent the multidrug resistance phenotype^{7,21,22}. Preliminary findings of elevated amounts of P-glycoprotein in some ovarian carcinomas of patients failing to respond to chemotherapy have already demonstrated the usefulness of the present monoclonal antibodies for this purpose¹⁶.

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Bombesin-like peptides can function as autocrine growth factors in human small-cell lung cancer

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The autocrine hypothesis proposes that a cell produces and secretes a hormone-like substance that can interact with specific membrane receptors on its surface to induce effects such as proliferation¹. Thus, a cancer cell could act to stimulate its own growth. Bombesin and bombesin-like peptides (BLPs) such as gastrin-releasing peptide (GRP) cause various physiological responses in mammals², including stimulation of proliferation of 3T3 mouse fibroblasts³ and normal human bronchial epithelial cells *in vitro*⁴ and induction of gastrin cell hyperplasia and increased pancreatic DNA content *in vivo* in rats^{5,6}. Human small-cell lung cancer (SCLC) cell lines produce and secrete BLPs⁷⁻¹² and can express a single class of high-affinity receptors for BLPs¹³. Exogenously added BLPs can also stimulate the clonal growth and DNA synthesis of SCLC *in vitro*^{14,15}. These findings suggest that BLPs function as autocrine growth factors for this tumour. One way to test this hypothesis is to interrupt the function of the endogenously produced BLPs. Here, we demonstrate that a monoclonal antibody to bombesin binds to the C-terminal region of BLPs, blocks the binding of the hormone to cellular receptors and inhibits the clonal growth of SCLC *in vitro* and the growth of SCLC xenografts *in vivo*. These results demonstrate that BLPs can function as autocrine growth factors for human SCLC.

The C-terminal region of bombesin and GRP, which competes effectively for the same class of high-affinity receptors on pituitary cells and SCLC membranes, is required for receptor binding and physiological activity^{13,16,17}. Murine monoclonal antibodies were prepared against a synthetic analogue of amphibian bombesin (Lys 3-bombesin) which has the same C-terminal heptapeptide sequence as human and porcine GRP^{18,19}. The neuropeptide substance P has the same carboxy-terminal Leu-Met dipeptide as bombesin but is rarely found in SCLC and binds to a different set of receptors than bombesin^{7,16}. Thus, we screened for monoclonal antibodies that bound to bombesin but not substance P. One anti-bombesin monoclonal antibody, 2A11 (IgG₁ κ), showed no cross-reactivity with substance P and was purified from ascites for study.

Using solid-phase synthetic peptides as targets in a radioimmunoassay (RIA), the 2A11 antibody bound to bombesin, the Lys 3 derivative of bombesin used for immunization, the Tyr 4 derivative of bombesin used for iodination, GRP 1-27, GRP 14-27 and GRP 20-27, whereas GRP 22-27, GRP 1-16, substance P and other tachykinins²⁰ showed insignificant 2A11 binding despite some C-terminal homology with bombesin and GRP (Table 1). In addition, a series of unrelated peptides that can be produced by SCLC, such as calcitonin, adrenocorticotrophic hormone (ACTH), arginine vasopressin (AVP) and neurotensin did not bind 2A11. Note that, although SCLCs make BLPs, the 2A11 antibody did not bind to live, intact SCLC (Table 1).

We next used 2A11 to develop a quantitative, competitive RIA system for BLPs using solid-phase 2A11 and ¹²⁵I-labelled Tyr 4-bombesin. This assay was able to detect BLPs in the range of 5-100 pg per well and BLPs were detected by the RIA in extracts of all 10 SCLC lines tested (Table 2). These values are in good agreement with previously reported quantitative data on many of the same tumour lines using rabbit anti-bombesin heteroantisera⁷. In contrast, none of the non-SCLC lines

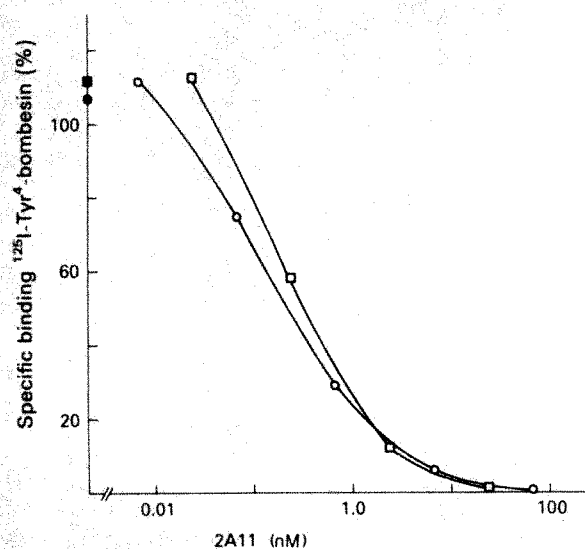


Fig. 1 Inhibition of binding of labelled bombesin in a radioreceptor assay by monoclonal antibody 2A11 using rat brain membranes (□)¹⁶ or small-cell lung cancer (NCI-H345) cell (○) preparations¹³. The black symbols on the ordinate show that 67 nM MOPC 21 control immunoglobulin did not inhibit the binding of the labelled peptide to either receptor preparation.

Methods. ¹²⁵I-Tyr 4-bombesin (50–80 μ Ci μ g⁻¹, 40,000 c.p.m. per test) was incubated with the indicated concentrations of purified monoclonal antibody 2A11 for 60 min at 25°. Receptor binding assays were conducted using 10 mg wet weight of crude rat brain homogenate or 2×10^6 cells of SCLC line NCI-H345 as described previously^{16,30}. The difference in binding in the absence and presence of an excess unlabelled bombesin (1 μ M) was taken as a measure of specific binding.

(adenocarcinoma, large-cell and squamous cell lung carcinoma) or the non-lung cell lines (B-lymphoblastoid, T-cell lymphoma and melanoma) expressed detectable levels of BLPs (<0.01 pmol mg⁻¹ acid soluble protein) (Table 2).

As 2A11 bound to the C-terminal region of BLPs (such as GRP 14–27 and GRP 20–27), we tested the ability of the antibody to block the binding of labelled bombesin to its cell-surface receptor on small-cell lung cancer and rat brain membranes (Fig. 1). Antibody 2A11 blocked bombesin–receptor interaction in a dose-related fashion with both receptor preparations (Fig. 1). Almost complete inhibition of bombesin binding (using 12–18 nM labelled bombesin) occurred with 10 nM 2A11. An indifferent mouse myeloma protein, MOPC 21, of the same isotype as 2A11 (IgG₁ κ) at 67 nM did not significantly affect bombesin–receptor interaction ($<1\%$ inhibition).

Antibody 2A11 was then examined for its effect on the *in vitro* growth of SCLC cell lines using an agarose cloning assay. Two types of SCLC cell lines were tested against various concentrations of 2A11. The first was a 'classic' SCLC line (NCI-N592) with typical SCLC morphology (growth in tight balls in suspension, scant cytoplasm) and high specific activities of L-dopa decarboxylase²¹. The second was a 'variant' SCLC line (NCI-N417) that deviates morphologically (growth as loose chains, with large cell cytology and abundant cytoplasm) from typical SCLC, has undetectable levels of L-dopa decarboxylase, grows rapidly, clones with high efficiency and is amplified for the *c-myc* oncogene^{21,22}. Both cell lines produce detectable levels of bombesin (Table 2) and both have the SCLC features of high specific activities of the BB isozyme of creatine kinase and neurone-specific enolase^{21,23}. The clonogenic growth of both cell lines was markedly inhibited by $\geq 3 \mu$ g ml⁻¹ 2A11 (Table 3). In contrast, 10μ g ml⁻¹ of the isotype-matched mouse myeloma protein MOPC 21 or boiled 2A11 showed no significant inhibition of SCLC growth in the same assay (Table 3). Exogenously added synthetic bombesin can stimulate the clonal growth of SCLC in serum-free medium (Table 3)¹⁴. The addition of 50 nM of

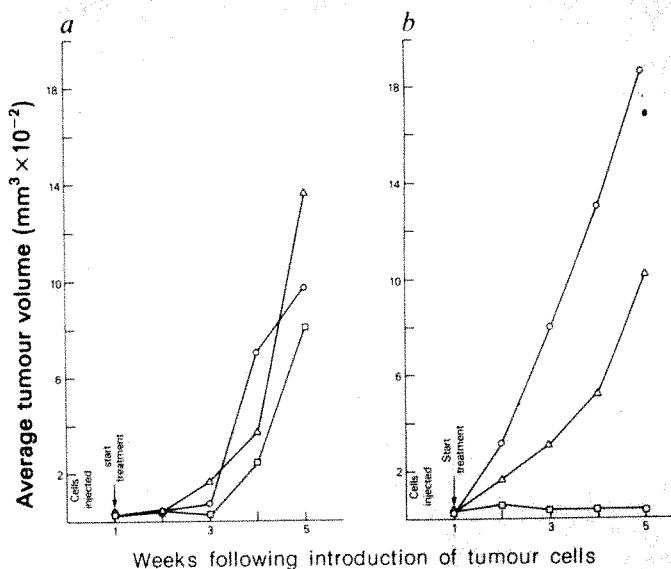


Fig. 2 Effect of treatment with monoclonal antibody 2A11 (□), control monoclonal antibody MOPC 21 (Δ) or PBS (○) on growth of human tumour xenografts in athymic nude mice. *a*, Human malignant melanoma cell line NCI-H234A; *b*, human small-cell lung cancer line NCI-N592.

Methods. Tumour cells (10^7 /mouse) were subcutaneously injected into the flanks of athymic nude mice (BALB/c *nu/nu*) and a palpable mass formed after 7 days. Treatment begun on day 7 consisted of three trial groups (5 mice per group), each given 0.5 ml i.p. injections three times a week of either PBS (○), 200 μ g purified MOPC 21 immunoglobulin (Δ) or 200 μ g of purified 2A11 antibody (□). Thus, each mouse in the immunoglobulin-treated groups received a total of 600 μ g immunoglobulin per week for four weeks. Tumours were measured once a week. Tumour growth is reported as an average relative tumour volume, s.e.m. $\pm 10\%$.



Fig. 3 Representative nude mice 5 weeks after xenografting with small-cell lung cancer NCI-N592 and treated with MOPC 21 (mouse 1), PBS (mouse 2) or 2A11 (mouse 3). Arrows point to the site of tumour innoculation. Large tumours are seen for the two controls (1 and 2) and no tumour mass was detected in mouse 3 at week 5.

exogenous synthetic bombesin added simultaneously with 2A11 completely prevented the 2A11 growth inhibition demonstrating the specificity of the 2A11 effect (Table 3).

Nude mouse heterotransplant studies were used to investigate the ability of 2A11 to inhibit SCLC growth *in vivo*. Two tumour cell lines were used in this study, the classic SCLC line NCI-N592 and a melanoma cell line, NCI-H234A, which did not make BLP and which was unresponsive to bombesin *in vitro*¹⁴, to serve as a control. One week after their inoculation into nude

Table 1 Immunological cross-reactivity of solid-phase synthetic peptides with bombesin using the 2A11 binding assay

Peptide target	Cross-reactivity (%)
Bombesin-like peptides	
Bombesin (BN)	100
Lys 3-BN	98
Tyr 4-BN	96
Gastrin-releasing peptide (GRP 1-27)	102
GRP 14-27	102
GRP 20-27	112
GRP 1-16	0.26
GRP 22-27	<0.02
Tachykinins	
Substance P, physalaemin, kassinin, elodeisin	all <0.02
Unrelated peptides	
Calcitonin, vasoactive intestinal peptide, motilin, β -endorphin, leu-enkephalin, glucagon, somatostatin, neurotensin, xenopsin, ACTH, AVP	all <0.07
Bovine serum albumin (BSA)	<0.02
Live small-cell lung cancer cells	<0.02

BALB/c mice were immunized subcutaneously three times with Lys 3-bombesin conjugated to BSA²⁴ (100 μ g bombesin equivalents per injection; the first dose was in complete Freund's adjuvant, the last two in incomplete Freund's). Monoclonal antibodies were derived by standard methods following fusion to the mouse myeloma cell line X63-Ag8.653 (refs 25, 26). Hybrids were screened for anti-bombesin activity 10 days after fusion by indirect RIA using radio-autography on solid-phase target plates coated with bombesin, substance P or 1% BSA. Hybridomas producing antibodies binding to bombesin but not BSA or substance P were cloned several times. Monoclonal antibody 2A11 (IgG₁ κ) was grown in ascites, purified²⁷ and used for all subsequent tests. For solid-phase RIA, peptides (all purchased from Peninsula Laboratories) were adsorbed to 96-well polyvinyl chloride microtitre plates (Dynatech) by adding 0.05 ml 10 μ g ml⁻¹ peptide in PBS for 1 h, washing in PBS, then blocking with a 1% BSA/PBS solution. Porcine sequence synthetic GRP peptides were used¹⁸. Viable SCLC cell lines NCI-H128, NCI-H209 and NCI-H345 at 10⁵ cells per well were tested in binding assays using 96-well plates with glass filters (V & P Scientific, San Diego)²⁸. Purified 2A11 (10 μ g ml⁻¹) was added, incubated and washed three times. Protein A affinity purified rabbit anti-mouse IgG (Cappel Laboratories) (1.6 μ g ml⁻¹) was added, incubated, washed three times, then ¹²⁵I-protein A (Pharmacia, labelled by chloramine T method to 40–50 μ Ci μ g⁻¹) 40,000 c.p.m. per well was added, incubated, washed eight times and the wells cut out and counted in a Beckman γ -counter. All incubations were for 1 h at room temperature in PBS with 1% BSA in 0.025 ml volumes. Percentage cross-reactivity = (c.p.m. ¹²⁵I-protein A bound on peptide target – c.p.m. bound on BSA target)/(c.p.m. bound by bombesin – BSA background $\times$ 100). The bombesin target caused 2A11 binding sufficient to bind 33,000 c.p.m. of ¹²⁵I-protein A whereas the BSA background bound 30 c.p.m. Mouse myeloma protein MOPC 21 (IgG₁ κ , Litton Bionetics) was used as a control at 10 μ g ml⁻¹ and did not bind to the peptides. All values are the average of triplicate determination and variation between replicates was <5%.

mice each cell line produced a palpable tumour mass. The established tumours were then subjected to three experimental treatments (five mice each group) consisting of: (1) phosphate-buffered saline (PBS); (2) the isotype-matched immunoglobulin MOPC 21; and (3) 2A11. Treatment was administered by intraperitoneal (i.p.) injection three times weekly and tumour growth was monitored as a function of tumour volume over the next four weeks of therapy (Fig. 2). The melanoma cell line, NCI-H234A, showed unhampered tumour growth in all three experimental groups with each group of mice attaining an average tumour volume of 1,000 mm³ by week 5 (Fig. 2a). In contrast, dramatic suppression of SCLC growth (NCI-N592) was observed following 2A11 treatment (tumour mass in all mice <50 mm³) while control groups of MOPC 21- and PBS-treated mice demonstrated rapid tumour growth (tumour mass in all mice >1,000 mm³ by five weeks) (Figs 2b; 3). Note that in MOPC 21-treated controls there was an unexplained tem-

Table 2 Quantitation of BLPs in human cell lines using a competitive RIA with monoclonal antibody 2A11

Cell line providing extract	Immunoreactive BLP (pmol per mg acid-soluble protein)
Small-cell lung cancer lines	
NCI-H128	18.4
NCI-H209	17.4
NCI-H345	7.8
NCI-H510	5.4
NCI-N592	3.9
NCI-H187	3.7
NCI-H69	2.3
NCI-H146	1.6
NCI-N417	0.95
NCI-H446	0.13
NCI-H82	0.04
Non-small-cell lung cancer lines	
A549 (adenocarcinoma)	<0.01
NCI-H23 (adenocarcinoma)	<0.01
NCI-H125 (adenocarcinoma)	<0.01
NCI-H157 (large cell lung cancer)	<0.01
9812 (large cell lung cancer)	<0.01
U1752 (squamous cell lung cancer)	<0.01
Other human cell lines	
NCI-H128BL (B-lymphoblastoid)	<0.01
HCI-H78 (T-cell lymphoma)	<0.01
NCI-H234A (malignant melanoma)	<0.01

Cell lines were grown as described previously^{21,23} and for preparation of cell extracts for RIA, 1–5 $\times$ 10⁷ cells were washed three times in PBS and resuspended in 1 ml of 2 M acetic acid, homogenized, heated in a boiling water bath for 15 min, clarified by centrifugation at 3,000 r.p.m. and the supernatant lyophilized and stored at –80°. The freeze-dried extract was resuspended in 0.5–1 ml PBS, reclarified and protein concentration determined using a BioRad assay kit. Dilutions of this extract were tested in a quantitative competitive RIA. Antibody 2A11 was adsorbed to 96-well polyvinylchloride plates at 500 ng per well followed by saturation with 1% BSA-PBS. Unlabelled bombesin (covering a range of 1–1,000 pg per well bombesin for a standard curve) or cell extract peptides at different dilutions were added to triplicate wells and incubated for 1 h at 25 °C. Without removing the unlabelled peptides, 12,000 c.p.m. of ¹²⁵I-Tyr 4-bombesin (labelled by the chloramine-T method to 50–80 μ Ci μ g⁻¹) was added to each well and incubated overnight at 4 °C, washed eight times and counted. All additions were in 1% BSA-PBS in 0.025-ml volume, and the washes were with 1% BSA-PBS. GRP 1-27, GRP 14-27 and GRP 20-27 were equipotent with bombesin in competing for labelled bombesin and the midpoint of the assay curve detected 25 pg bombesin per well. In contrast, substance P, unrelated peptides and synthetic analogues of bombesin or GRP with substitutions blocking binding to the cellular BLP receptor were unable to significantly compete with labelled bombesin in this assay. In addition, MOPC 21 immunoglobulin did not bind the labelled peptide. Using the standard curve, the BLP equivalent of the cell extract peptides was determined and expressed as pmol of BLP mg⁻¹ of acid soluble protein in the extract. Results are the average of triplicate determinations with variation between replicates of <5%.

porary delay in tumour growth in some mice, but in all cases a large tumour had formed by five weeks.

In the 2A11-treated group, three more weeks of treatment were given; two mice never showed tumour growth, but three developed progressively growing NCI-N592 tumours despite 2A11 treatment. These antibody-resistant tumours are under study. At week 5, the 5 mice with large NCI-N592 tumours in the PBS-treated group were then treated with 2A11 antibody for three weeks and the tumours in all 5 mice stopped growing and became necrotic.

In conclusion, we have reported on a monoclonal antibody to amphibian bombesin that cross-reacts with BLPs of human origin. The antigenic determinant for this antibody has been localized at the C-terminal region of bombesin and GRP. We have demonstrated that 2A11 can be used in an RIA for the quantitative detection of BLPs in extracts of human SCLC. The antibody was shown to block bombesin-receptor interaction in

Table 3 The effect of monoclonal antibody 2A11 on the *in vitro* clonal growth of human small-cell lung cancer lines

Addition to cloning medium	Cell line	
	NCI-N592	NCI-N417
Growth as % control (no of colonies)		
Experiment 1 ($\mu\text{g ml}^{-1}$)		
None	100 (30)	100 (583)
2A11 0.1	93 (28)	98 (571)
2A11 1.0	83 (25)	96 (560)
2A11 3.0	0 (0)	3 (18)
2A11 10	0 (0)	1 (7)
MOPC 21 10	97 (29)	99 (577)
Experiment 2		
None	100 (32)	100 (610)
50 nM bombesin	>100 (664)	>100 (820)
2A11 10 $\mu\text{g ml}^{-1}$	0 (0)	5 (30)
MOPC 21 10 $\mu\text{g ml}^{-1}$	94 (30)	100 (607)
Boiled 2A11 10 $\mu\text{g ml}^{-1}$	88 (28)	97 (593)
2A11 10 $\mu\text{g ml}^{-1}$ + bombesin 50 nM	>100 (340)	>100 (910)

Tumour cell cloning assays were performed in serum-free HITES medium as described previously²⁹. HITES medium contains RPMI-1640 (GIBCO) supplemented with 10 nM hydrocortisone, 5 $\mu\text{g ml}^{-1}$ bovine insulin, 10 $\mu\text{g ml}^{-1}$ human transferrin, 10 nM 17 β -estradiol and 30 nM selenium. A single-cell suspension of NCI-N592 or NCI-N417 cells taken from log-phase cultures was mixed with 0.3% agarose in HITES (v/v) in the absence or presence of purified 2A11 or MOPC 21 antibody, bombesin, boiled antibody or 2A11 + bombesin (added simultaneously) and plated over a pre-hardened base layer of 0.5% agarose/HITES. The NCI-N417 culture plates were supplemented with 0.1% BSA which greatly enhanced clonal cell growth. NCI-N592 cells were seeded at 5×10^4 per plate, whereas NCI-N417 cells were plated at 1×10^4 . Plates were pre-screened to verify single-cell distribution of test cells and scored 21 days later for colony formation. Cell aggregates of >50 cells were scored positive for colony growth. All studies were done in triplicate and each point represents the mean colony count. For all studies the s.e.m. was $\pm 10\%$.

both brain and SCLC membrane preparations. Finally, purified antibody when added to an *in vitro* tumour cloning assay or to an *in vivo* nude mouse xenograft assay prevented tumour cell growth. The antibody effect *in vitro* was blocked by synthetic bombesin, and the antibody did not inhibit the growth of a melanoma tumour cell line that did not make BLPs. We conclude that BLPs can indeed function as autocrine growth factor(s) for human SCLC. It is possible that the bombesin stimulation of growth seen in SCLC could reflect a physiological role of BLPs in some cells during normal fetal growth and development. In addition, our studies with monoclonal antibody 2A11 against BLP offer a new immuno-hormonal approach of manipulating the growth of human SCLC in patients. Although these studies used monoclonal anti-peptide antibodies to inhibit BLP action, it is possible that growth suppressive effects could be obtained using bombesin antagonists or anti-receptor antibodies.

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Translocation of the p53 gene in t(15;17) in acute promyelocytic leukaemia

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Recent studies have demonstrated that the cellular tumour antigen p53 (ref. 1) can complement activated *ras* genes in the transformation of rat fibroblasts, suggesting that the gene encoding p53 may act as an oncogene^{2,3}. Here, by using *in situ* chromosomal hybridization^{4,5}, we have mapped the p53 gene to human chromosome 17, at bands 17q21-q22, the region containing one of the breakpoints in the translocation t(15;17) (q22;q21) associated with acute promyelocytic leukaemia (APL)^{6,7}. Hybridization of p53 and *erb-A* (17q11-q12) probes to malignant cells from three APL patients indicated that the p53 gene is translocated to chromosome 15 (15q+), whereas *erb-A* remains on chromosome 17. Analysis of variant translocations demonstrates that the 15q+ chromosome contains the conserved junction⁸, suggesting a role for p53 in the pathogenesis of APL. However, rearrangements of the p53 gene were not detected on Southern blotting of DNA from leukaemic cells of four APL patients with t(15;17).

To determine the chromosomal localization of the p53 gene, we performed *in situ* chromosomal hybridization using a p53-specific probe. We used an ³H-labelled complementary DNA clone, pp53-176, containing the entire coding region (1.35 kilobases, kb) of the murine p53 gene⁹, for *in situ* hybridization to normal human metaphase chromosomes prepared from phytohaemagglutinin-stimulated peripheral blood lymphocytes. Figure 1 shows the distribution of labelled sites in 100 metaphase cells examined. This hybridization resulted in labelling of a specific region of a single chromosome, namely, the long arm of chromosome 17. Of 100 metaphase cells examined, 24 (24%) were labelled on region q2, bands q21-q25, of one or both chromosomes 17. A total of 183 grains was observed; thus, the labelled sites on region q2 of chromosome 17 represented 14.2% of all labelled sites (26/183). The largest number of grains was clustered at 17q21-q22 (18/183, 10%, $P < 0.005$).

The localization of the p53 gene to 17q21-q22 is of particular interest because of the nonrandom abnormalities involving this

Table 1 *In situ* hybridization of a p53 probe to metaphase cells with a t(15;17)

Patient	No. of metaphase cells analysed	Total no. of labelled sites	No. of labelled sites (%)			
			Normal 15	15 q+	Normal 17	17 q-
1	100	141	5 (3.5%)	14 (9.9%)*	10 (7.1%)†	2 (1.4%)
2	50	73	1 (1.4%)	7 (9.6%)*	7 (9.6%)*	0 (—)
3	63	84	2 (2.4%)	7 (8.3%)†	10 (12%)*	1 (1.2%)

* χ^2 values correspond to $P < 0.05$.† χ^2 values correspond to $P < 0.20$; however, all grains were clustered on the translocated material from chromosome 17, at bands q21-q22.

chromosome in human tumours. Gain or loss of a whole chromosome 17 has been a frequent finding in haematological malignant diseases as well as in various solid tumours, and structural rearrangements involving 17q are especially frequent in the leukaemias¹⁰. In particular, a reciprocal translocation involving chromosomes 15 and 17 at bands q22 and q21, respectively, is a characteristic abnormality in APL. We have observed this translocation in each of 42 patients with APL that we studied in our laboratory (ref. 7 and our unpublished data).

With regard to the t(15;17), the proto-oncogene *erb-A* has also been mapped to the long arm of chromosome 17. Analysis of hybridizations of an *erb-A*-specific probe to metaphase cells with a t(15;17)¹¹, and Southern blot analysis of somatic cell hybrids retaining the 15q+ chromosome¹², showed that *erb-A* remains on the rearranged chromosome 17. Moreover, no rearrangements of this gene were detected on digestion with eight restriction enzymes of genomic DNAs from five APL patients¹³.

To determine whether the p53 gene or *erb-A* is localized near the chromosomal breakpoint on chromosome 17 that is involved in the t(15;17), we hybridized the two probes to metaphase cells from three APL patients who had this rearrangement. Figure 2 shows the distribution of labelled sites, using each probe, on the rearranged chromosomes and on the normal homologues in cells from patient no. 1. Of 100 metaphase cells examined after hybridization with the p53 probe, 10 (10%) were labelled on the normal chromosome 17; these sites were clustered at 17q21-q22, and represented 7.1% (10/141) of all labelled sites ($P < 0.05$). Two labelled sites were observed on the 17q- chromosome. In 14 metaphase cells, labelled sites were observed on the 15q+ chromosome; they were clustered at bands q21-q22 of the translocated chromosome 17 (14/141, 9.9%, $P < 0.05$). The chromosome 15 homologue had only five labelled sites dispersed along the long arm (3.5%, $P > 0.05$). Thus, the p53 gene, which is normally localized at 17q21-q22, is translocated to chromosome 15 as a result of the t(15;17). Analysis of cells from the two other patients with this rearrangement gave similar results (Table 1).

In contrast, an analysis of metaphase cells hybridized to *erb-A* revealed that this gene remains on the 17q- chromosome and thus is located proximal to the t(15;17) breakpoint (Fig. 2). In 100 metaphase cells examined from patient no. 1, 11 and 10 labelled sites were observed on the normal chromosome 17 and on the 17q- chromosome, respectively (normal 17: 11/125, 8.8%; 17q-: 10/125, 8.0%, $P < 0.05$). In each case, the grains were clustered at 17q11-q12. Two labelled sites were noted on the normal chromosome 15 homologue (1.6%), and four were observed on the 15q+ chromosome (3.2%, $P > 0.05$). Similar results were obtained for hybridizations of metaphase cells from the two other APL patients. This result is consistent with the findings of previous studies in which both *in situ* chromosomal hybridization and Southern blot analysis of DNA from somatic cell hybrids retaining the 15q+ chromosome, were used^{11,12}. Recent results of *in situ* hybridizations to metaphase chromosomes derived from a fibroblast cell line with a different translocation, t(15;17)(q22;q11), suggest that *erb-A* is localized between bands 17q11 and q21 (ref. 13). Our results indicate that the breakpoint on chromosome 17 in the t(15;17) in APL is bracketed by the *erb-A* and p53 genes.

To determine whether the p53 gene is structurally altered by this translocation, we subjected DNA from four APL patients with the t(15;17) to Southern blot analysis using the mouse p53 cDNA probe; human placental DNA served as a germline control. It was necessary to reduce the stringency for these hybridizations, as there is only ~80% homology between the mouse and human genes¹⁴. DNA of high relative molecular mass was prepared and digested with the appropriate restriction enzyme, electrophoresed in 0.8% agarose, and blotted onto Gene Screen Plus transfer membranes (NEN). The blots were hybridized with the plasmid pp53-176, which had been nick-translated with ³²P to a specific activity of 3×10^8 d.p.m. μg^{-1} . Hybridization was for 16 h at 37 °C in 50% formamide, $5 \times \text{SSC}$ ($1 \times \text{SSC} = 0.15 \text{ M}$ sodium chloride, 0.015 M trisodium citrate), 10% dextran sulphate, 1% SDS, 20 mM sodium phosphate (pH 6.8), 0.1% Ficoll, 0.1% polyvinylpyrrolidone, 0.1% bovine serum albumin, and denatured salmon sperm DNA at $100 \mu\text{g ml}^{-1}$. After hybridization, the filters were washed in $3 \times \text{SSC}$, 0.1% SDS for 3 h at 55 °C, followed by 30–120 min at 68 °C, as needed, to reduce the background. In all four patients, we observed the following germline bands: *Eco*RI, 18 kb; *Pst*I, 2.9 and 1.6 kb; and *Pvu*II, 4.0 kb. This pattern is consistent with previously published results, except that the 1.6-kb band expected with *Pvu*II¹⁴ was not observed consistently, suggesting that hybridization of this region to the murine probe was weaker than to the human probe. Thus, there were no detectable changes in the structure of the p53 gene in these samples.

Recent studies with the human APL cell line HL60 have demonstrated that most of the p53 gene has been deleted from these cells, except for a small amount of residual 5' sequences¹⁵. From our *in situ* chromosomal hybridizations and Southern analyses, it is clear that there are no major deletions in the p53 gene in fresh APL cells. Our inability to detect new restriction fragments of the p53 gene on Southern blots, however, does not

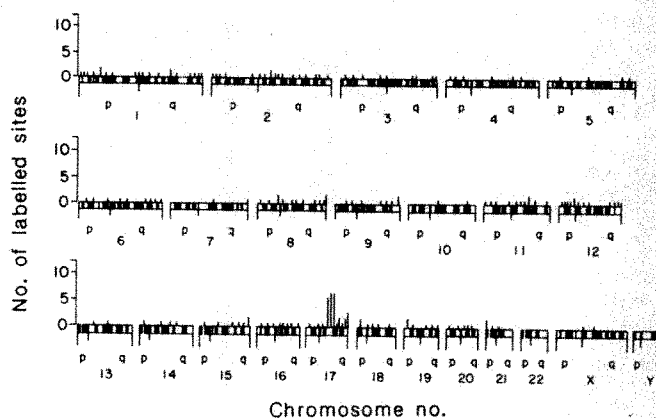


Fig. 1 Distribution of labelled sites in 100 normal metaphase cells. From a total of 29 grains on chromosome 17, 18 (62%) were localized at 17q21-q22. Human metaphase cells prepared from phytohaemagglutinin-stimulated peripheral blood lymphocytes were hybridized with ³H-labelled pp53-176 plasmid DNA nick-translated with all four ³H-labelled deoxynucleotide triphosphates to a specific activity of 2.8×10^7 d.p.m. μg^{-1} . *In situ* hybridization was performed as described previously⁵.

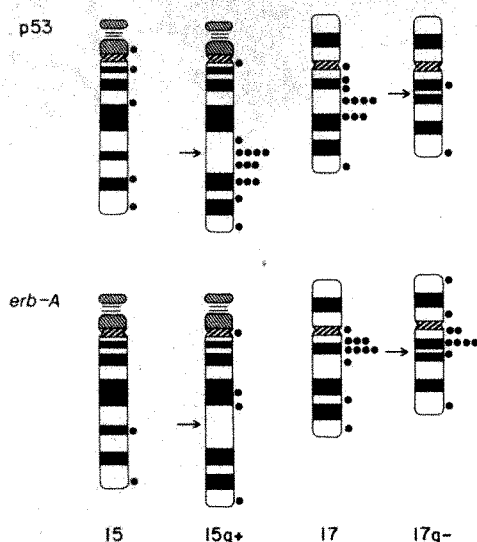


Fig. 2 Distribution of labelled sites on normal chromosome homologues 15 and 17, and on the translocation derivatives 15q+ and 17q-, in metaphase cells from an APL patient (no. 1) with a t(15;17), which were hybridized to the p53 or *erb-A* probes. One hundred metaphase cells were examined for each probe. Arrows identify the breakpoint junctions on the rearranged homologues. The results of these hybridizations indicated that the p53 gene is translocated to chromosome 15, whereas *erb-A* remains on chromosome 17. The radiolabelled probes were nick-translated to specific activities of 2.9×10^7 (p53) and 4.9×10^7 d.p.m. μg^{-1} (*erb-A*). The *erb-A*-specific probe (c-HerbA-1) was a 1.6-kb human *erb-A* cDNA clone¹¹.

exclude the possibility that the gene is activated by the translocation, as a rearrangement may have occurred outside the regions homologous to the probe (protein-coding region). Such a situation has been reported for the *c-abl* proto-oncogene in the t(9;22) associated with chronic myelogenous leukaemia¹⁶. Similarly, in Burkitt's lymphoma, rearrangements are usually seen within the introns of the protein-coding regions of *c-myc* in the common t(8;14); however, such rearrangements occur outside this region in the variant translocations t(2;8) and t(8;22)^{17,18}. Thus, in the latter translocations, rearrangements of *c-myc* cannot be detected with a probe containing only protein-coding sequences.

The location of two genes (the p53 gene and *erb-A*), each with transforming capabilities, adjacent to the t(15;17) breakpoint raises the question as to which gene is involved in the pathogenesis of APL. In a reciprocal translocation such as t(15;17) there are two rearranged junctions, and there is no *a priori* reason for choosing either the 15q+ or the 17q- chromosome as containing the critical junction. The analysis of complex translocations that involve three chromosomes can reveal which of the junctions that are seen in the simple translocation is conserved. Such an analysis has been performed for t(15;17) as well as t(8;14) and t(9;22)⁸; in the latter two translocations, the 14q+ and 22q- (Philadelphia, Ph¹) chromosomes contained the conserved junctions. Subsequent molecular analysis has shown that each of these chromosomes contains the critical genes [*c-myc* and immunoglobulin heavy chain on 14q+ (refs 17, 18), and *c-abl* and *ber* (breakpoint cluster region) on Ph¹ (refs 16, 19)]. For t(15;17), the 15q+ chromosome junction was conserved in three clearcut complex translocations^{20,21}. In two other reports of variant translocations, however, either the karyotypes permit several interpretations²² or they have not been published²³.

Thus, most of the data available support the notion that the 15q+ chromosome contains the critical junction, and this would suggest that the p53 gene has a critical involvement in the transformation of normal myeloid cells into leukaemic cells whose maturation is arrested at the promyelocyte stage. Further investigation of the role of the p53 gene in t(15;17) awaits

molecular cloning of the junction fragment and analysis of the p53 gene product in these malignant cells.

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Note added in proof: We have recently confirmed the localization of p53 to 17q12-q24 by hybridizing a human p53 cDNA probe to normal metaphase cells. In addition, a smaller yet significant cluster of grains was observed on 17p11-p13; this labelling was not seen with the mouse probe.

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Unusual organization and diversity of T-cell receptor α -chain genes

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T lymphocytes recognize cell-bound antigens in the molecular context of the self major histocompatibility complex (MHC) gene products through the surface T-cell receptor(s). The minimal component of the T-cell receptor is a heterodimer composed of α and β subunits, each of relative molecular mass (M_r) $\sim 45,000$ (refs 1-3). Recently, complementary DNA clones encoding these subunits have been isolated and characterized along with that of a third subunit of unknown function, termed γ (refs 4-9). These studies revealed a primary structure for each subunit that was clearly similar to that of immunoglobulin and indicated a somatic rearrangement of corresponding genes that are also immunoglobulin-like. Recently, the analysis of the sequence organization of the T-cell receptor β -chain¹⁰⁻¹⁶ and T-cell-specific γ -chain gene families¹⁷ has been reported. We now present an initial characterization of the murine T-cell receptor α -chain gene family, and conclude that although it is clearly related to the gene families encoding immunoglobulins¹⁸, T-cell receptor β -chains¹⁰⁻¹⁶ and also T-cell γ -chains¹⁷, it shows unique characteristics. There is only a single constant (C) region gene segment,

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which is an exceptionally large distance (~20–40 kilobases (kb) in the cases studied here) from joining (*J*) gene segments. In addition, the *J* cluster and the variable (*V*) segment number seem to be very large. Finally, in the case studied here, a complete α -chain gene shows no somatic mutation and can be assembled directly from V_α , J_α and C_α segments without inclusion of diversity (D_α) segments.

We reported previously⁶ the isolation of a cDNA (pHDS58) from a cloned cytotoxic T lymphocyte (2C) that encodes the T-cell receptor α -chain¹⁸. The sequence of this cDNA clone showed that, by analogy with immunoglobulin genes, the cDNA can be divided into *V*, *J* and *C* regions⁶. The whole α -cDNA, or its fragments containing one or more regions, was used to probe *Eco*RI-digested BALB/c embryo DNA. The results can be summarized as follows (Fig. 1). First, there are at least four fragments (20, 15, 9 and 2.2 kb) carrying V_{2C} or V_{2C} -like sequences, indicating that the pHDS58 *V* region is derived from a family of closely homologous germline *V* DNA segments. Second, the 2C *V* probe hybridized most intensely with the 2.2-kb fragment, suggesting that this fragment contains the germline counterpart of the pHDS58 *V* region. Third, the pHDS58 *J* region is encoded in an *Eco*RI fragment of ~800 base pairs (bp). Fourth, *Eco*RI fragments of 6.2 and 3.9 kb contain the genetic information for the 3' and 5' ends of the *C* region, respectively, suggesting that, unlike the β - and γ -genes, the BALB/c embryo contains only one *C* gene segment for the α -chain. In addition, when we analysed BALB/c embryo DNA using *V*-region probes derived from several other α -chain cDNAs, we detected different hybridization patterns composed of multiple bands (data not shown), suggesting that, unlike the β -chain gene family, the α -chain gene family contains many germline V_α DNA segments.

The 2.2-kb *Eco*RI fragment containing the germline counterpart of the pHDS58 V_α region was cloned (clone λ 2.2) and sequenced. The sequence is identical to that derived from the corresponding region of pHDS58, indicating that somatic mutation did not occur in the formation of the complete α gene (Fig. 2a). The same conclusion has been reached for a β -chain¹² and for T-cell γ genes¹⁷.

DNA clones containing the various genomic DNA sequences of the J_α - C_α region in the germline configuration are summarized in Fig. 3a (see legend for details of the derivation of these genomic clones). All genomic clones isolated with phage λ vectors are from BALB/c and the cosmid clone 11E is from C57BL/6. However, the Southern blot analysis (Fig. 3b) indicates that there is no gross length polymorphism between these two strains in the relevant region.

We determined the exon-intron structure of the C_α sequence by DNA sequencing (Fig. 2c). Apart from the overall homology with the *C*-gene segments of immunoglobulins, T-cell receptor, β -chain and T-cell-specific γ genes, a striking feature of the α -chain *C* region is its shortness. This is primarily reflected in the first exon which encodes a *C* domain of only 87 amino acids. By contrast, the analogous exons of the T-cell receptor β -chain genes and of the immunoglobulin heavy-chain genes and T-cell γ genes are, respectively, 124 (ref. 10) and 110 amino acids^{17,19}. The second *C*-segment exon is similar to that of the T-cell receptor β -chain and T-cell γ -gene *C* segments in encoding only a short, cysteine-containing peptide of similar size, but not homologous in sequence, to the exon that encodes the hinge region of IgG²⁰. The third exon primarily encodes the putative transmembrane domain and the proposed cytoplasmic tail, and the fourth exon encodes only 3' untranslated region. Terminal exons that encode only untranslated region are a feature of some class II MHC genes²¹, but are without precedent among immunoglobulin, T-cell receptor β -chain^{10,11} and T-cell γ genes¹⁷.

The *J*-segment sequence (Fig. 2b) derived from clone λ *9.1 (Fig. 3) clearly represents the *J* segment used to assemble the α -chain gene (pHDS58) active in 2C cells. It demonstrates two features that are highly conserved among *J* segments: the nucleotide sequence GGNNNGGN that encodes 'G-G',

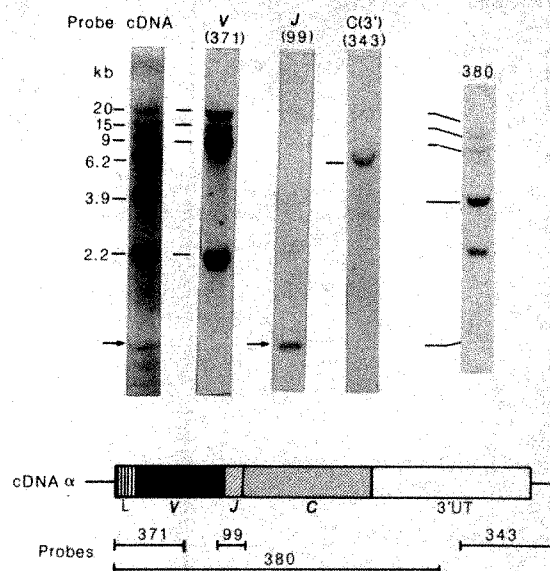


Fig. 1 *Eco*RI-cleaved BALB/c embryo DNA electrophoresed through 0.9% agarose gels, transferred to nitrocellulose (BA85)²², and hybridized to probes derived from cDNA pHDS58 as indicated in the diagram. Probes were labelled by nick translation²³, and the sizes (kb) of the fragments detected are indicated. All samples, except those probed with probe 380, were run on the same gel, which was subsequently divided into strips. The arrow indicates a fragment of ~800 bp, which is detectable with *J* probe (no. 99) and faintly with other *J*-containing probes. Fragment sizes were determined from co-electrophoresis of phage λ DNA cut with *Hind*III and *Sall*.

which is invariant in functional *J* segments outside the γ -gene family, and the presence 5' to the *J*-coding region of the conserved nonamer and heptamer sequences implicated in *V*-*J* rearrangement (Fig. 4)²². The sequences, separated by 12 bp, are essentially complementary to a heptamer and nonamer that occur immediately 3' to the V_α segment separated by 23 bp (Figs 2a, 4). The spacings of 23 bp in the sequences 3' to V_α and of 12 bp in the sequences 5' to J_α are common among the three T-cell-specific rearranging genes, α , β and γ , and suggest (according to an empirical rule^{23,24}) that these *V* segments can join directly to the respective *J* segments without the involvement of the third (*D*) segment (see below).

By the criteria of the conserved 'G-G' coding triplets and the conserved heptamer and nonamer sequences, we searched for additional *J* segments and found a second (J_1) *J* segment >700 bp downstream of the first one, and a third one (J_2) ~0.5 kb downstream of the second (Figs 2b, 3a). A fourth, J_{C10} used by the α -chain of a helper T-cell hybridoma, was mapped by Southern blotting to ~4.0 kb downstream of J_2 . Three more *J* segments (J_{C11} , J_{B8C3} and J_{DFL12}) were mapped on another genomic clone, λ 8.2, whose DNA occupies a region upstream of the C_α gene segments (Fig. 3a). Thus, we have mapped seven different J_α segments over a distance of ~20 kb. More *J* segments may exist in the 19-kb region between J_{DFL} and C_α , and possibly also 5' to J_{2C} . By comparison, the two T-cell receptor J_β clusters are spread over 1.1 and 1.9 kb (refs 10, 12), the immunoglobulin J_H cluster over 1.36 kb (refs 23, 25) and the immunoglobulin J_K cluster over 1.4 kb (refs 22, 26). In each of these cases the *J* segments are relatively evenly spaced ~50–500 bp apart. Given that the first seven J_α segments we have identified were all different and that our analysis already points to clustering of some J_α segments (J_{2C} , J_1 and J_2 ; and J_{C11} , J_{B8C3} and J_{DFL}), the α -gene family must contain many more J_α segments. If there are 20 J_α segments, the probability that the first seven analysed would be different is significant (30%). Our analysis of *J* segments shows those used by cytotoxic T lymphocytes and by T-helper cells to be interspersed one with another, as with T-cell receptor β -chain segments^{10,12}, implying

Fig. 2 Comparison of T-cell receptor α -chain sequences and cDNA α -sequences. *a*, Comparison of germline V sequence (gV_{α}) determined using clone λ 2.2 as described in ref. 34 and cDNA pHDS58 sequence⁶ throughout the V segment. The perfect homology of the two sequences is indicated (for the protein coding regions only) by dashes, but extended upstream of that for all the available pHDS58 sequence⁶. A candidate transcriptional start site is superscripted with an arrow, being within a sequence (underlined) resembling Py---PyAPyPyPyPy³⁵. Between 20 and 30 bp upstream of this is a candidate TATA box (underlined)³⁵. The exon *V.I* denotes the putative leader region although the transcript of this region is contiguous with that of upstream untranslated sequence. The splice donors and receptors are overlined with dots. The proposed heptamer and nonamer V segment signal sequences at the 3' end of V are indicated. *b*, Comparison of germ line J_{α} sequence (gJ_{α}) and pHDS58 sequence through the J segment. The J_{α} segment completes the α -chain V region, that is, exon *V.II*. 3' of this is shown sequence of another putative J_{α} segment (J_1) from downstream in the germline. *c*, Comparison of germline C_{α} sequence (gC_{α}) and pHDS58 sequence throughout the C segment. Homology is shown by dashes only for the protein coding sequences. Exon *C.IV* encodes only untranslated RNA. The germline sequence of this continues for over 500 bp with the same sequence as was determined for pHDS58 (data not shown).

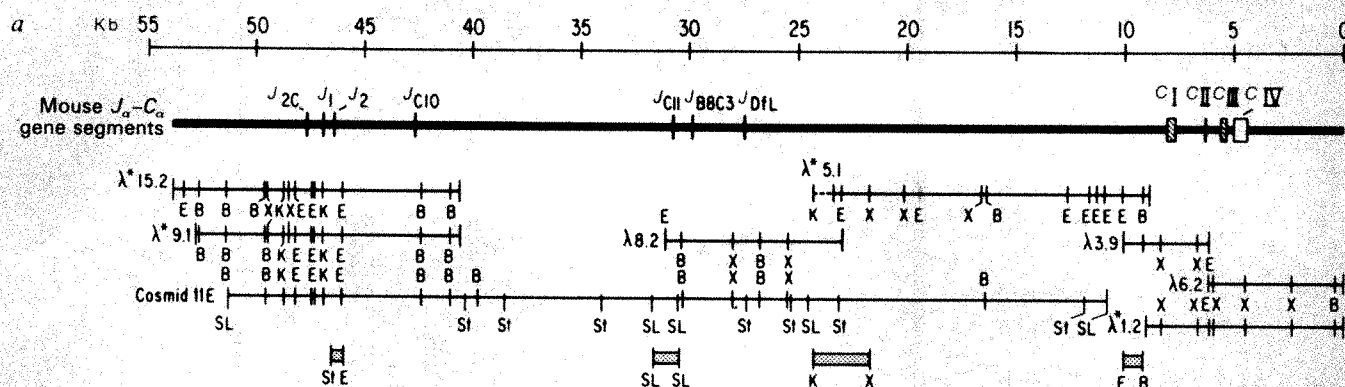


Fig. 3 The murine embryonic *J-C* region. *a*, The map was deduced by analysis of the recombinant clones indicated. Clones $\lambda 3.9$ and $\lambda 6.2$ were derived from a library of six-fractionated BALB/c embryo *EcoRI* fragments using probes 380 and 343 (see Fig. 1), respectively. Clone $\lambda 8.2$ derived from the same library using a *J* probe from a cDNA α derived from T_H hybridoma B8C3 (L. Glimcher and P. Allen, manuscript in preparation). Clones $\lambda *1.2$, $\lambda *5.1$ were derived from a Charon 4A library of partially *EcoRI**-digested BALB/c embryonic DNA using the 800-bp *EcoRI/BamHI* fragment EB from $\lambda 3.9$ as the hybridization probe. Clone $\lambda *15.2$ and $\lambda *9.1$ were derived from the same library using probe 99 (Fig. 1). Cosmid 11E was derived from a partial *Sau3A* library of C57.B6 embryo DNA. The maps of the clones, assignment of coding sequences and overlap between clones were determined by restriction analysis, cross-hybridization and DNA sequence, and were confirmed by genomic Southern analyses of embryonic DNA of BALB/c and of C57.B6 mice. As an example, the probe KX, stretching from a *KpnI* site in the L arm of Ch4A to an *XbaI* site in clone $\lambda *5.1$, detects in the murine germ line a *BamHI* fragment of 10.2 kb and an *XbaI* fragment of 3.8 kb, consistent with the mapping shown here. Furthermore, the *BamHI/EcoRI* fragment of $\lambda 8.2$ in turn hybridized to KX, as well as to the 400-bp fragment that lies between the left-most *EcoRI* site in the insert of $\lambda *5.1$, and the L-arm. Restriction sites are: E (*EcoRI*), B (*BamHI*), X (*XbaI*), K (*KpnI*), SL (*SalI*) and St (*Sst*). Maps are not necessarily complete, notably for *EcoRI* and *XbaI* on cosmid 11E. In addition to B8C3, J_α segments were localized for T_H hybridomas C10 and C11 (Glimcher and Allen, in preparation), and for cytotoxic T-lymphocyte clone DFL.12 (ref. 36). Exons are boxed and shaded if translated. Germline $V_{2C\alpha}$, cloned in $\lambda V2.2$, lies upstream at an as yet undetermined distance from J_{2C} . *b*, Southern analysis of BALB/c and C57.B6 genomic DNA using cosmid 11E-derived probes. Embryonic DNA from BALB/c and C57.B6 mice was co-electrophoresed on 1.2% agarose gels after restriction with *Sst* (St) or *KpnI* (K) and *SalI* (SL). Southern transfers of the electrophoresed DNA were probed with either the 1.2-kb *SalI* fragment (dotted line in *a*) for St-cut DNA or the 0.6-kb St-E fragment (dotted line in *a*) in the case of SL-K-cut DNA. Methods are further described in Fig. 1 legend.

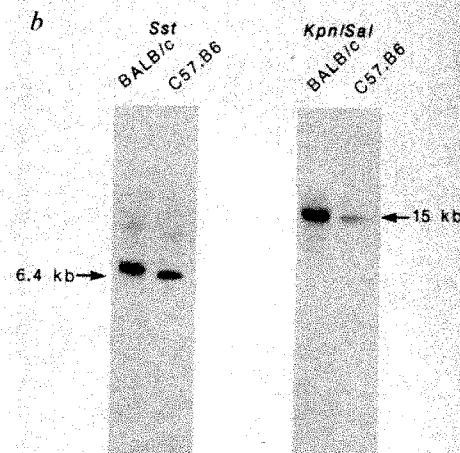
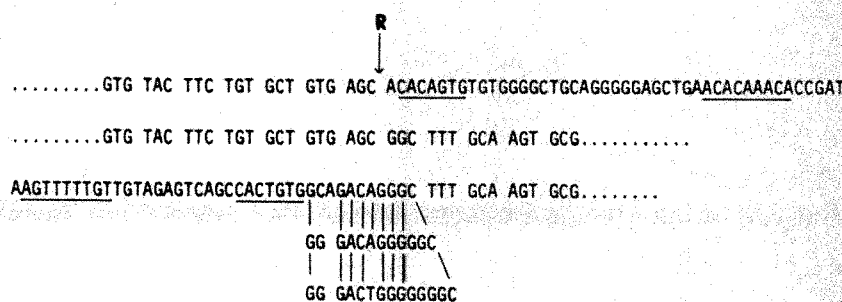


Fig. 4 Comparison of germline $V_\alpha(gV_\alpha)$, germline $J_\alpha(gJ_\alpha)$ and cDNA pHDS58 sequences indicates recombination of V_α to J_α at point R. The germline gJ has homology at its 5' end to sequences of $\delta B1-1$ and $\delta B2-1$ (refs 13, 14). The proposed heptamer and nonamer sequences 3' to V and 5' to J, respectively, are underlined.



that there is no functional clustering of *J* segments in either gene family.

One consequence of the large *J* cluster is the presence of an unusually long intron between *V-(D)-J* and *C* segments in at least some assembled α genes. For example, in the 2C α gene this intron is ~40 kb, which is without precedent among immunoglobulin or immunoglobulin-like genes characterized to date. Very large introns have recently been discovered in the *Ubx* and *Antp* loci of *Drosophila melanogaster*²⁷; in *Drosophila*, the large intron may be differentially processed, whereas here the primary function of the large intron may be to accommodate many *J* segments. Because of the similarity between both the gene segments that comprise T-cell receptor and immunoglobulin genes and the overall organization of these gene families, transcriptional activity at the promoter of a rearranged V_α gene segment may be enhanced in an analogous manner to that of rearranged immunoglobulin V_H and V_K segments. However, if a tissue-specific enhancer (analogous to the immunoglobulin enhancer) does exist in the *J-C* intron of the T-cell receptor α -chain genes, it must be activating the promoter

associated with the rearranged V_α gene segment at a distance of at least 20 kb in 2C cells. Alternatively, the existence of such a large J_α cluster may have excluded the activation of V_α segment transcription by this mechanism.

One interesting feature of the J_{2C} segment is its unusual length of 63 bp (Fig. 2b)^{10,12,25}. At its 5' end, the *J* sequence GCAGACAGGGC resembles the sequences of the T-cell receptor β -chain gene *D* segment^{13,14} (Fig. 4). Thus, the J_{2C} segment can be regarded as a *D*-type element of 11 bp pre-fused in the germ line to a *J*-type element of 52 bp. In both IgH and T-cell receptor β -gene families, single *D* elements occur in relatively close proximity to the respective *J* clusters^{13,14,28}. It is possible that the analogous D_α segment does not exist, having already fused to J_α to generate the J_{2C} segment in the germ line. The implication is that, albeit at low frequency, cells other than lymphocytes can effect *D-J* recombination.

Another interesting feature of the J_{2C} segment is its direct joining with the germline V_{2C} segment in 2C cells. The fusion of the germline V_{2C} and J_{2C} segments should create a novel V_α probe reactive *EcoRI* fragment of 1.9 kb in 2C cells (probe 371)

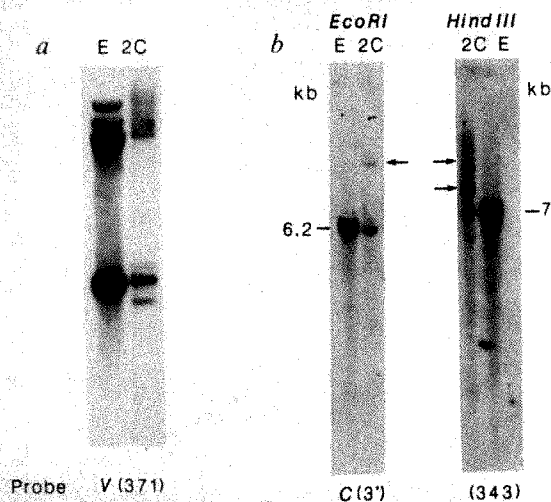


Fig. 5 *a*, *EcoRI*-cleaved DNA of BALB/c embryo, E and cytotoxic T-lymphocyte clone 2C cells, electrophoresed through 0.9% agarose, and analysed as described in Fig. 1. *b*, *EcoRI*-cleaved and *HindIII*-cleaved DNA of BALB/c embryo, E and 2C cells, analysed as described in Fig. 1 and hybridized with probe 343 (see Fig. 1). Bands strongly detected in 2C DNA but not in embryo DNA are arrowed.

that would not be present either in germline DNA, B-cell DNA or DNA of T cells that have not made this particular *V-J* combination. Such a fragment is detected in 2C DNA (Fig. 5a). In IgH, T-cell receptor β -chain or T-cell γ -gene families, extra nucleotides are commonly found between the fused *V* and *J* segments¹⁸. These insertions are either template-independent or the result of the participation of a third type of germline DNA segment, *D*, in the fusion process^{13,14,28,29}. Although the 12/23-bp spacer rule permits direct *V-J* joining for the T-cell β -receptor and T-cell γ -gene families, no such case has yet been clearly demonstrated. By contrast, the germline *V_{2C}* and *J_{2C}* sequences presented here can fully account for the sequence of pHDS58 in the *J* region (Fig. 4), strongly suggesting that a direct *V-J* joining has taken place. However, α -cDNA sequences derived from other cytotoxic T-lymphocyte clones and helper T-cell hybridomas (unpublished data) as well as the sequences of other germline *J_α* segments strongly suggest that complete α genes are also assembled with the participation of *D* segments.

Probe 343 (Fig. 1) derives solely from the 3' end of *C_α*, and detects in BALB/c embryo DNA a single *EcoRI* fragment of 6.2 kb and a single *HindIII* fragment of ~7 kb. Surprisingly, when this probe is directed against *EcoRI*- or *HindIII*-cleaved 2C cell DNA, it detects additional fragments (Fig. 5b) which, because of their reproducibility, are apparently not caused by partial digestion of 2C cell DNA. The nature of the gene rearrangement in 2C cells relative to the BALB/c germ line that generates these novel fragments is unclear. Candidates include chromosomal rearrangements 3' to the *C_α* locus, or amplification of germline sequences which, when in single copy, are undetectable with probe 343. Note that in the experiments shown in Fig. 5b, an excess of digested BALB/c embryo DNA was loaded in an effort to detect in the germline single-copy, unrearranged versions of the novel bands present in 2C cells.

In conclusion, our characterization of murine gene segments encoding the T-cell receptor α -chain indicates a multi-gene family that is comprised of many *V*-gene segments, a large array of *J*-gene segments and a single *C_α* gene. Furthermore, our data strongly suggest that a given pair of *V_α* and *J_α* segments can join either directly, without the participation of a *D_α* segment, or indirectly, through one or more *D_α* segment(s). Thus, the scope for combinatorial diversity of the α -gene family seems to be significantly greater than that of IgH, IgK and T-cell receptor β - and γ -gene families. By contrast, the data presented here for the 2C α gene and those reported earlier by others for β genes¹²

and by us for γ genes¹⁷ did not give any indication of somatic mutation, an important source of diversity for immunoglobulin genes¹⁸. This apparent lack of somatic mutation in T-cell receptor genes may be related to the relatively large combinatorial diversity seen in the α -gene family, the latter partially compensating for the former. However, apparent somatic mutation of both α - and β -chain T-cell receptor genes was noted on *in vitro* passage and subcloning of an interleukin-2-secreting T-cell hybridoma³⁰. The role of somatic mutation in diversifying the T-cell receptor repertoire³¹ remains an open question.

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Genomic organization of the genes encoding mouse T-cell receptor α -chain

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The vertebrate immune system uses two kinds of antigen-specific receptors, the immunoglobulin molecules of B cells and the antigen receptors of T cells. T-cell receptors are formed by a combination of two different polypeptide chains, α and β (refs 1-3). Three related gene families are expressed in T cells, those encoding the T-cell receptor, α and β , and a third, γ (refs 4-6), whose function is unknown. Each of these polypeptide chains can be divided into variable (*V*) and constant (*C*) regions. The *V_β* regions are encoded by *V_β*, diversity (*D_β*) and joining (*J_β*) gene segments that rearrange

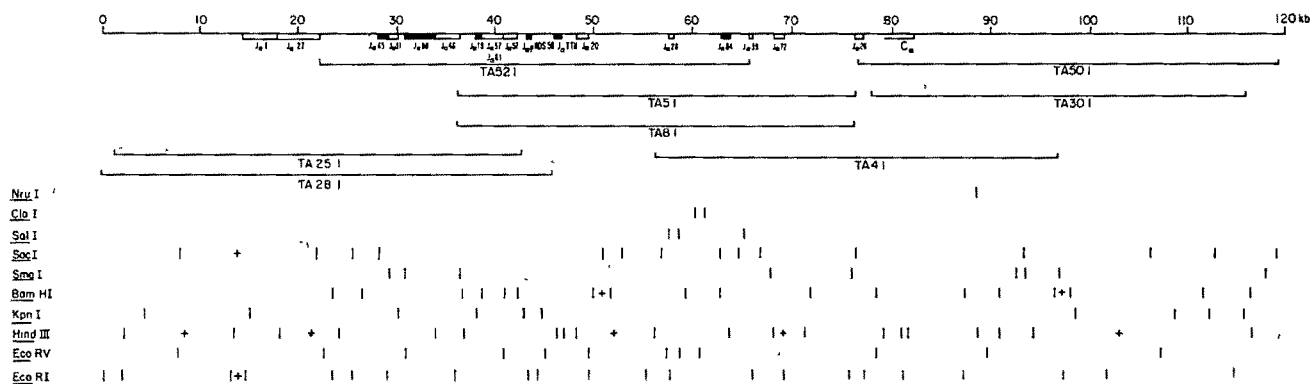


Fig. 1 Restriction map of the mouse cosmid cluster containing the J_α gene segments and the C_α gene. Open boxes indicate restriction fragments that hybridize to the 500-bp *Sau3A* fragment C_α probe from A10 cDNA clone or to the synthetic oligonucleotide J_α probes. Solid boxes indicate the J_α gene segments that have been sequenced in this paper. Unassigned sites between two restriction sites are indicated by +. The J_α gene segments lie to the 5' side of the C_α gene whose direction of transcription is to the right. The J_α gene segments are named according to the cDNA clone from which they originated^{15,16,18}. The cDNA library was constructed in λ gt10 vector as described³⁸, with minor modification. The second-strand synthesis was done using RNase H, DNA polymerase I and T4 ligase³⁹ and the unincorporated nucleotides were removed at every step by NH_4 -acetate/ethanol precipitation. The cosmid library was constructed and screened as described previously⁴⁰. The oligonucleotides for J_α gene segments were purified in a 20% preparative polyacrylamide gel. The DNA band was visualized by placing a silica gel 60F-254 precoated TLC plate (EM reagents) under the gel and shining short ultraviolet light from above. The DNA band was then cut out, crushed and mixed with 3 ml 0.5 M NH_4 -acetate, 10 mM Mg-acetate, 1 mM EDTA, 0.1% SDS and elution was at 37 °C for 3 h with shaking. The acrylamide was then separated from the DNA by spinning in a Quick-sep Isolab column and the aqueous solution containing oligonucleotide was concentrated to <0.2 ml by several *sec*-butanol extractions. Desalting of DNA was done in a 5 ml Sephadex G25 column. Fractions were collected and monitored by a spectrophotometer. The pure oligonucleotides were kinased as described previously⁴¹. Hybridization of the J_α oligonucleotides was done in 6×SSC, 5×Denhardt's, 0.1% SDS and 100 $\mu\text{g ml}^{-1}$ denatured salmon sperm DNA at 37 °C with 5×10^5 c.p.m. ml^{-1} . The blots for oligonucleotides were washed at 25 °C in 6×SSC, 0.1% SDS.

in the differentiating T cell to generate V_β genes⁷⁻¹⁴. The V_γ regions are encoded by V_γ , J_γ and, possibly, D_γ gene segments^{4,5}. Studies of α complementary DNA clones suggest that α -polypeptides have V_α and C_α regions and are encoded by V_α and J_α gene segments and a C_α gene¹⁵⁻¹⁷. Elsewhere in this issue we demonstrate that 18 of 19 J_α sequences examined are distinct¹⁸, indicating that the J_α gene segment repertoire is much larger than those of the immunoglobulin (4-5) or β (14) gene families. Here we report the germline structures of one V_α and six J_α mouse gene segments and demonstrate that the structures of the V_α and J_α gene segments and the α -recognition sequences for DNA rearrangement are similar to those of their immunoglobulin and β -chain counterparts. We also show that the J_α gene-segment organization is strikingly different from that of the other immunoglobulin and rearranging T-cell gene families. Eighteen J_α gene segments map over 60 kilobases (kb) of DNA 5' to the C_α gene.

A full-length cDNA probe, A10, was obtained for the isolation of germline V_α , J_α and C_α clones from a λ gt10 cDNA library of the T-cell helper hybridoma 1.9.2 using an oligonucleotide probe specific for the C_α gene^{15,16}. DNA sequence analysis showed that the A10 clone was identical in sequence to the published full-length cDNA clone TT11 derived from a T-cell hybridoma¹⁵. A V_α -specific probe was derived from the A10 cDNA and used in Southern blot analysis to demonstrate a V_α rearrangement in the BW5147 tumour DNA (data not shown). As both α -sequences come from different T-cell hybridomas which have the BW5147 cell line as a common parent, these observations suggest that clone A10 is derived from the T-cell tumour BW5147 α -gene. This V_α -specific probe was then used to isolate both germline and rearranged clones of the BW5147 V_α subfamily from a λ genomic library of the 1.9.2 hybridoma DNA.

A C_α -specific probe was obtained from the A10 cDNA clone and used in Southern blot analysis of several mouse DNA clones digested with various restriction enzymes. These data indicate that there is only one cross-hybridizing C_α gene (data not shown). The C_α -specific probe was then used to screen a cosmid library constructed from liver DNA of the inbred B10.D2-H-2^{dml} mouse to obtain three overlapping cosmid clones (Fig. 1). Two successive chromosomal walks were then performed with single-copy probes isolated from the 5' ends of cosmid clones TA4.1 and TA52.1 to yield five additional clones (Fig. 1). These eight

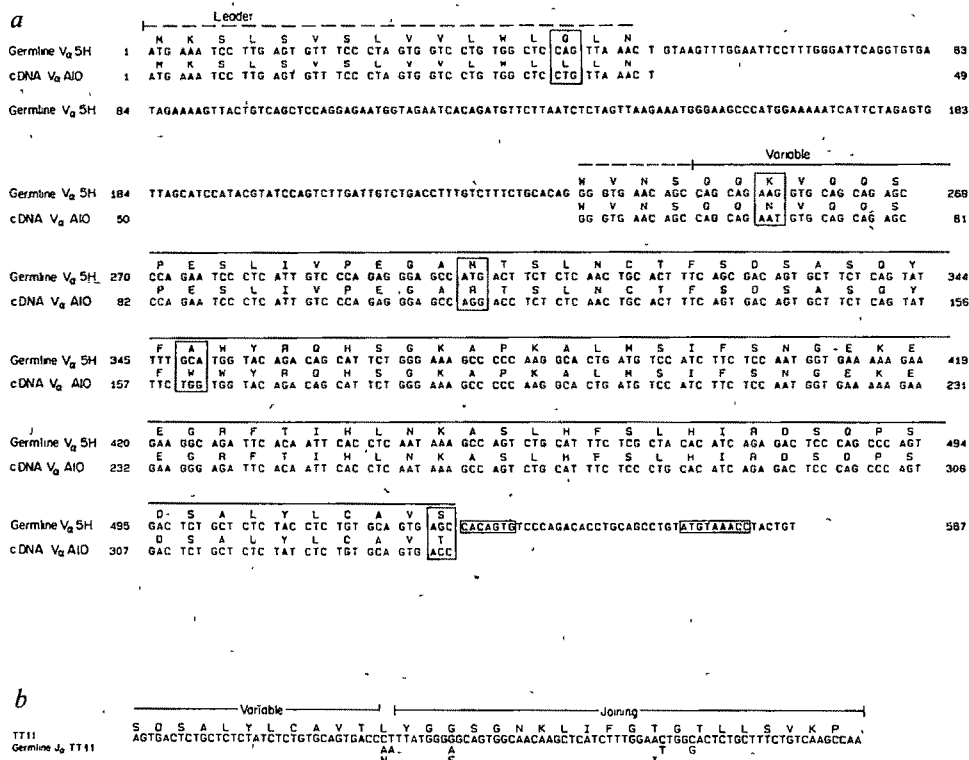
cosmid clones encompassed 119 kb of DNA and contained all 18 J_α gene segments described elsewhere in this issue¹⁸.

To study the structure of the V_α gene segment, we isolated a germline V_α clone, V_α 5H, using the V_α -specific probe from the A10 cDNA. The V_α 5H has a leader exon of 49 nucleotides separated by an intron of 188 nucleotides from a V exon of 287 nucleotides (Fig. 2a). The leader coding segment is split by an intron probably four codons from its 3' end. The V_α 5H sequence seems to represent a functional V_α gene segment and belongs to the V_α 1 subfamily which contains at least 10 different members (ref. 18 and A.W., unpublished data). The V_α 5H coding region is 96% homologous at the DNA level and 98% homologous at the protein level to the coding region of the A10 cDNA clone, hence these sequences represent closely related members of the V_α 1 subfamily (see ref. 18).

The 3' end of the V_α gene segment is marked by a recognition sequence for DNA rearrangement¹⁹. It consists of a conserved heptamer sequence CACAGTG, a 21-base pair (bp) spacer sequence and an A+T-rich nonamer sequence (Fig. 2). The recognition signal is very similar to those of the κ and heavy (H) immunoglobulin¹⁹ and β and γ T-cell gene families^{5,7,8,10,11}.

The C_α gene is 79 kb from the 5' end of the cosmid cluster and 37 kb from the 3' end (Fig. 1 and A.W., unpublished data). The locations of J_α gene segments were determined using 18 oligonucleotide probes that were synthesized corresponding to the 5' or 3' ends of the J_α cDNA sequences^{15,16,18}. As the 5' and 3' ends of the J_α coding regions are different from one another¹⁸, the oligonucleotide probes do not cross-hybridize in the conditions used. In each case only one restriction enzyme fragment from the cosmid clones hybridized to each of the individual oligonucleotide probes. Eighteen J_α gene segments map over a region which extends from 3 to 63 kb 5' to the C_α gene (Fig. 1). The precision in the assignment of the J_α gene segment locations varies within 0.5 to 4 kb of DNA because of the limited number of restriction enzymes that we have used to map the cosmid clones. Hence, the distribution of J_α gene segments is strikingly different from the distribution of the J gene segments of immunoglobulin and the T-cell β - and γ -genes. The J_α gene segments are spread over at least 60 kb of DNA, whereas the J_β and J_γ gene clusters extend over only 2 kb of DNA. Assuming that the 18 J_α gene segments analysed are a random representation of the J_α gene segment repertoire, the fact that all of them

Fig. 2 a, DNA sequence of the germline and the rearranged V_{α} gene segments; **b**, alignment of germline and rearranged sequences of the V - J junction. **a**, The leader and variable coding regions of the germline V_{α} 5H and rearranged A10 cDNA clone are indicated. See text for a discussion of 5' and 3' boundaries of the V gene segment. Boxes in the coding region indicate codons with replacement changes and boxes in the 3' end of the V_{α} gene segment indicate the recognition sequence for DNA recombination. The A10 cDNA clone was obtained from the 1.9.2 helper T-cell cDNA library using an oligonucleotide specific for the C_{α} region^{15,16}. The clone was subcloned in M13mp18 and sequenced by the dideoxy chain termination method as described elsewhere^{20,42}. The λ genomic library from the 1.9.2 helper T-cell hybridoma was constructed in EMBL3 vector as described^{41,43}. The library was screened using a 300-bp *Rsa*I/*Eco*RI V_{α} -specific probe from A10 cDNA. The V_{α} -containing fragment from the V_{α} 5H λ clone was subcloned into M13mp18 or mp19 and sequenced as described^{20,42}. **b**, Upper line represents the TT11 cDNA sequence and the lower the germline J_{α} sequence. Above and below the nucleotide sequences are the translated protein sequences. V and J regions are indicated. Dots between the V and J region indicate extra nucleotides between them; dots below the nucleotide sequences indicate identity to the DNA sequence given above; a dash indicates identity to the protein sequence shown above. Only the 3' part of the V_{α} region of the cDNA is shown.



map to the available cosmid clones is consistent with the hypothesis that most of the J_{α} gene segments will map to the 63-kb region of DNA 5' of the C_{α} gene. As only one of 19 J_{α} sequences analysed in the accompanying paper¹⁸ was a repeat, the repertoire of J_{α} sequences is probably larger than 18.

Six germline J_{α} gene segments were subcloned into M13 bacteriophage and sequenced using specific oligonucleotide primers²⁰ (Fig. 3). The coding regions of the J_{α} sequences were identified by a sequence encoding Phe-Gly-X-Gly that is conserved in virtually all T-cell J gene segments. The 5' boundary of the J_{α} gene segments was determined by the presence of a one-turn recognition sequence for DNA rearrangement. The 3' boundary was determined by analyses of the germline and cDNA sequences¹⁸ in this region which permitted identification of the site for RNA splicing between the J_{α} and C_{α} sequences.

These are several unique features of the germline J_{α} segments. First, they are longer than the J gene segments of immunoglobulin and β -genes by 3–9 codons. Second, the J_{α} gene segments exhibit length heterogeneity at their 3' ends; this heterogeneity is in contrast to the other J gene segments within a particular family (λ , κ , H, β), which all have the same lengths at their 3' ends. All the immunoglobulin and T-cell J gene segments exhibit length heterogeneity at their 5' ends. Thus, the J_{α} sequences are more heterogeneous than their immunoglobulin and β counterparts¹⁸.

The recognition sequences for DNA rearrangement that lie 5' of each of the J_{α} gene segments are very similar to those found in the immunoglobulin and β -gene families (Fig. 3). They each have a highly conserved heptamer separated from an A+T-rich nonamer by a 12-bp spacer sequence. In addition, the 3' splicing site for joining the J_{α} to the C_{α} RNA sequences contains the canonical RNA splicing donor sequence GTAAG²¹.

The fact that the V_{α} gene segments have recognition sequences with 23-bp spacers (two DNA helix turns) and the J_{α} gene segments have recognition sequences with 12-bp spacers (one turn) establishes that all three rearranging gene families in T cells (α , β and γ) use similar kinds of recognition sequences for their V and J gene segments. In the case of the β -genes,

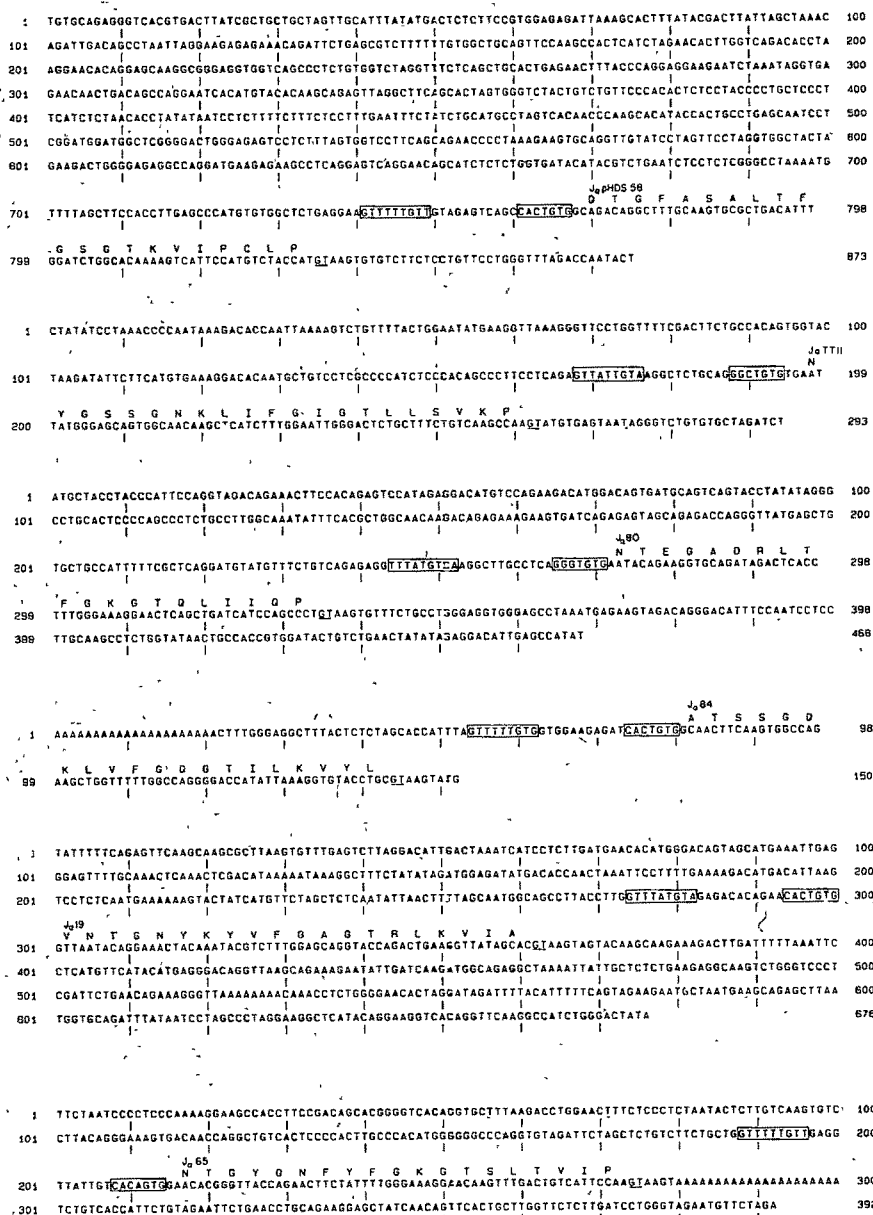
the D_{β} gene segments have a one-turn recognition sequence to the 5' end and a two-turn recognition sequence to the 3' end and accordingly join one- to two-turn recognition sequences in the process of rearrangement and juxtaposition of the V_{β} , D_{β} and J_{β} gene segments⁹. The question of whether or not D_{α} gene segments exist remains. However, even without D_{α} gene segments, a V_{α} may join to the J_{α} gene segment because of the complementary one- and two-turn recognition sequences.

The J_{α} gene segments are more widely spaced from one another than are their immunoglobulin and β -gene counterparts. The immunoglobulin and J_{β} gene segments within a cluster are separated by 36–519 nucleotides, with most of the J gene segments separated by <350 bp. We have sequenced 500 and 400 bp to the 5' and 3' side of the J_{α} 19 gene segment, 400 and 500 bp to the 5' and 3' sides of J_{α} 80, 400 bp to the 5' side of J_{α} 65 and 200 bp on both sides of the J_{α} TT11 gene segment. In addition, we have sequenced more than 900 bp 5' to the J_{α} pHDS 58 gene segment (Fig. 3 and A.W., unpublished data); in each case, we failed to observe an adjacent J_{α} coding region as defined by the invariant Phe-Gly-X-Gly sequence.

A comparison of homologous sequences from the J_{α} gene segments isolated from DNA of the B10.D2.H-2^{dml} mouse and the cDNA clones derived from AKR and BALB/c mice^{15,16} shows that these J_{α} sequences have several nucleotide differences as well as a one-codon sequence gap. Because each J_{α} oligonucleotide probe hybridizes to just a single germline sequence, these observations suggest that J_{α} gene segments are polymorphic. Analysis of V_{α} gene segments also reveals that there are many polymorphisms in the V_{α} gene segments¹⁸. The polymorphism that is present in the α -gene family is significantly greater than that seen in the β -gene family²².

The presence of a D_{α} gene segment in a rearranged V_{α} gene should lead to additional nucleotide sequence between the germline V_{α} and J_{α} gene-segment sequences. The alignment of the A10 cDNA clones and the available germline V_{α} and J_{α} gene segments demonstrates that there are two extra nucleotides between the V_{α} and J_{α} gene segments in this clone (Fig. 2b). The J_{α} gene segment is the germline equivalent to that expressed

Fig. 3 DNA sequence of six germline J_α gene segments. The J gene segments are translated into protein sequence with the one-letter code. The J_α designations are given above the protein sequence. Recognition sequences for recombination are boxed and the RNA donor splicing sites are underlined. Cosmid restriction fragments containing the J_α gene segments were purified using low-melting point agarose and then subcloned into M13 bacteriophage mp18 or mp19 or both⁴⁴. Plaques of M13 containing the J_α sequences in both orientations were identified by hybridization to the J_α oligonucleotides made from DNA sequences of both strands. The following cosmid fragments were used for subcloning: 0.5-kb *Hind*III fragment for J_α TT11, 1.8-kb *Kpn*I fragment for J_α pHDS 58, 2-kb *Xba*I fragment for J_α 80, 1-kb *Bam*HI/*Hind*III fragment for J_α 84, 2-kb *Bam*HI fragment for J_α 19 and 0.5-kb *Xba*I fragment for J_α 65. DNA sequencing by the dideoxy chain termination method was done essentially as described previously⁴² and modified²⁰. Some of the DNA sequencing was done by the chemical degradation method⁴⁵. DNA sequencing was done for both strands. For every J_α -containing fragment >0.5 kb, two complementary 17-mer J_α oligonucleotides were used for the first strand sequence analysis. The opposite strands of DNA were sequenced by two additional oligonucleotides (17-mer) made according to the information obtained in the first strand DNA sequencing. Hence, at least four different oligonucleotides were made and used for sequencing one J_α gene segment to obtain information on both strands. In one case in which we could not obtain clones containing opposite orientations of the J_α gene segment (J_α TT11), the sequence was confirmed by the Maxam-Gilbert sequencing method⁴⁵.



in the A10 cDNA, whereas the germline V_α gene segment belongs to the same V_α subfamily but is not identical. In the V_α , V_H and V_β gene families, members of the same V subfamily always end at the same codon position relative to the conserved second cysteine codon. The additional nucleotides between the rearranged V_α and J_α sequences may be explained in three ways. First, the extra nucleotides can arise from D_α gene segments that are joined to the V_α and J_α gene segments. Second, these extra sequences could arise as a result of N -region diversification. This mechanism results in the addition of random nucleotides to the 5' and/or 3' sides of the D gene segments during the joining process²³. In the immunoglobulin light-chain gene families, which do not have D gene segments, N -region diversity does not occur. Third, it is possible that the extra nucleotides are actually part of the V_α gene segment. These three possibilities cannot yet be distinguished. However, either D_α gene segments exist, N -region diversification occurs in a rearranging gene family lacking D gene segments, or the V_α gene segments within a subfamily differ in the lengths of their 3' ends.

The immunoglobulin and β -gene families use various mechanisms for somatic diversification. Junction variability occurs as a consequence of the flexibility in sites at which the gene segments are joined; and somatic hypermutation, the apparently random mutation of up to 3% of the bases in and

around a rearranged V gene, occurs in immunoglobulin genes, but rarely in β -genes^{24,25}.

The α -gene family also uses various mechanisms for somatic diversification. An alignment of the germline V_α and J_α gene segments with their cDNA counterparts revealed that there is flexibility in the sites at which the gene segments are joined together. For example, in the TA19 cDNA¹⁸, the J_α gene segment is joined five bases into the coding sequence. Thus, diversity can be introduced in the junctional region by a mechanism similar to those seen in the immunoglobulin and β -gene families. The data are inadequate to draw firm conclusions about somatic hypermutation in V_α genes because, apart from the A10 clone, these cDNA clones were derived from a thymic cDNA library. In B cells somatic hypermutation occurs late in B-cell development²⁶ and T cells in the thymus are at early stages of development. Two sets of α -gene segments do not exhibit somatic mutation. First, a comparison of the four germline J_α gene sequences analysed here with their cDNA counterparts from the same inbred strain reveals that there is no somatic mutation (see Fig. 3 and ref. 18). Second, three cDNA clones with identical V_α gene segments have been identified in the 19 different cDNA clones studied and all are found to be identical¹⁸.

The immunoglobulin and T-cell β - and γ -gene families have four distinct types of J -C organization (Fig. 4). (1) The immunoglobulin κ -genes have a cluster of four functional J

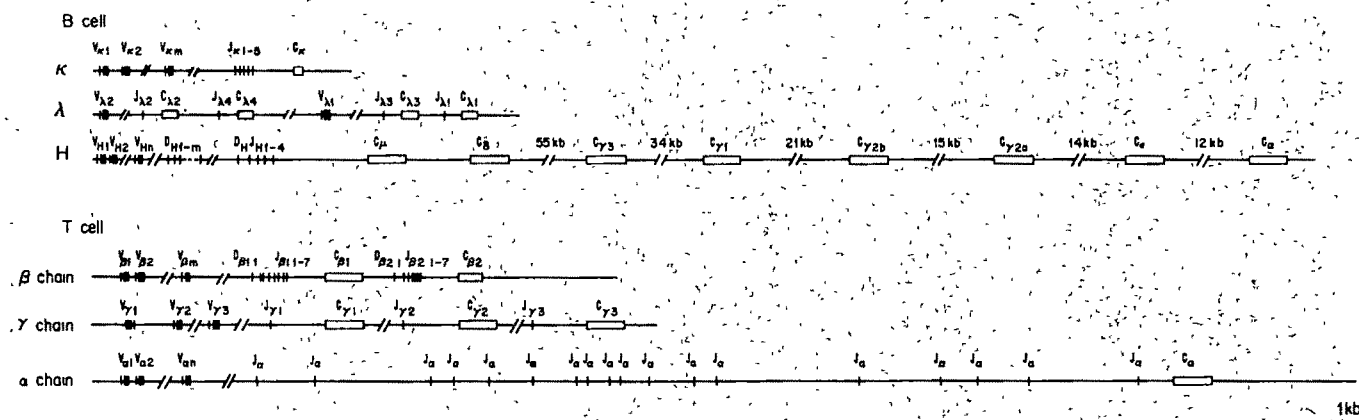


Fig. 4 Schematic diagram of the gene organization for the immunoglobulin and T-cell receptor gene families. Distances between *J* and *C* gene segments for immunoglobulin^{27,28,31,46} and T-cell rearranging^{5,7,10,11} families are indicated. Boxes indicate the *V* gene segments or the *C* genes, vertical lines the *D* or the *J* gene segments.

gene segments located 2.5 kb to the 5' side of a single *C_κ* gene^{27,28}; (2) the H-chain genes have four *J_H* gene segments lying 6.5 kb 5' to the *C_H* gene, the most 5' of eight different *C_H* genes^{29,30}; (3) the λ family has four single *J* gene segments, each lying 1.2–1.3 kb 5' of separate *C* genes³¹. Similarly, the γ-gene family has three single *J* gene segments, each lying 3.8 kb 5' of separate *C* genes⁵; (4) the β gene-family has two clusters of *J* gene segments, each with six functional members. These clusters are located ~2.5 kb to the 5' side of the two *C_β* gene segments^{7,10,11}. In each case, when multiple *J* gene segments exist, they are closely clustered, separated by 36–519 bp, with most of the *J* gene segments separated by <350 bp (Fig. 4). We have demonstrated here that the 18 *J_α* sequences map across 60 kb of DNA. As 18 out of 19 *J_α* gene segments analysed are different, many additional *J_α* gene segments will probably be found in this region. This is in striking contrast to the other five rearranging immunoglobulin families where the *J* gene segments are relatively close to the *C* gene (Fig. 4).

The disperse organization of the *J_α* gene segments has several consequences. (1) The size of nuclear transcripts for immunoglobulin and T-cell receptor genes is determined by the length of the *J*–*C* intron. In the immunoglobulin, β- and γ-gene families, these introns vary between 1.2 and 8 kilobases. In contrast, the homologous introns of the α-gene vary between 3 and >63 kb. Thus, the α-transcripts must vary from 6 to >66 kb. Several other eukaryotic genes have larger nuclear transcripts^{32–34}. (2) The large distance between the rearranged *V_α* gene and the *C_α* gene may have interesting regulatory implications. In immunoglobulin H-chain and κ light-chain genes, enhancer sequences are found in the intron between the 3'-most *J* and the *C* sequences^{35–37}. If similar enhancer sequences exist in the α-gene family, the enhancer sequence could be ≥60 kb from the promoter on which it presumably operates. This possibility raises interesting questions about the distances over which enhancers can operate. Alternatively, there may be multiple enhancer sequences, each associated with a *J_α* gene segment. (3) Three *J_α* gene segments, *J_α37*, *J_α57* and *J_α61*, are closely linked and seem to be most closely related to one another (see ref. 18). This observation suggests that the *J_α* gene segments fall into subfamilies, like the *V_α* gene segments. Hence, the duplication, expansion and diversification of *J_α* gene segments may also arise from unequal crossing-over, gene conversion and single base substitutions. (4) As T cells may not use somatic hypermutation²⁴, there seem to be compensatory mechanisms for increased diversification of the 3' ends of the *V_β* and *V_α* genes. In the β-genes this occurs in the increased repertoire of *J_β* gene segments and the extensive use of the *D_β* gene segments in all three translational reading frames²⁴. In the α-genes this occurs in the extensive *J_α* repertoire.

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In the accompanying papers, Hayday *et al.*⁴⁷ and Yoshikai *et al.*⁴⁸ have made similar observations concerning the organization of α genes in mice and humans, respectively.

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Organization and sequences of the variable, joining and constant region genes of the human T-cell receptor α -chain

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An essential property of the immune system is its ability to generate great diversity in antibody and T-cell immune responses. The genetic and molecular mechanisms responsible for the generation of antibody diversity have been investigated during the past several years^{1,2}. The gene for the variable (V) region, which determines antigen specificity, is assembled when one member of each of the dispersed clusters of V gene segments, diversity (D) elements (for heavy chains only) and joining (J) segments are fused by DNA rearrangement¹⁻³. The cloning of the β -chain of the T-cell antigen receptor^{4,5} revealed that the organization of the β -chain locus, which is similar to that of immunoglobulin genes⁶⁻¹² is also composed of noncontiguous segments of V⁷⁻⁹, D^{10,11}, J^{8,9,11} and constant (C) region genes⁶⁻¹². The structure of the α -chain seems to consist of a V and a C domain connected by a J segment¹³⁻¹⁶. We report here that the human T-cell receptor α -chain gene consists of a number of noncontiguous V and J gene segments and a C region gene. The V region gene segment is interrupted by a single intron, whereas the C region contains four exons. The J segments, situated 5' of the C region gene, are dispersed over a distance of at least 35 kilobases (kb). Signal sequences, which are presumably involved in DNA recombination, are found next to the V and J gene segments.

Southern gel analysis¹⁷ was performed using human genomic DNA and nucleotide probes corresponding to the V, J and C region sequences of a human T-cell receptor α -chain cDNA, pY14 (ref. 16). We find that the entire pY14 insert hybridizes to ~10 fragments when genomic DNA is digested with *Eco*RI, *Bam*HI, *Hind*III or *Bgl*II (Fig. 1). Using a C-region-specific probe or J-region-specific probe, only one or two of these bands can be assigned to the C and J sequences (data not shown), whereas the rest correspond to fragments homologous with V gene segments. These results suggest the presence of only one C region and multiple V gene segments.

Seventeen recombinant phage from a human genomic library (from ref. 18) carrying inserts that hybridized to the V region of pY14 (*V_αpY14.1*) under non-stringent hybridization conditions were isolated. The nucleotide sequence was determined for one clone (λV13). Figure 2 shows the nucleotide sequence of this V gene segment, *V_αpY14.2*, which is divided into two exons. The first exon stretches from amino acids 1 to 15 and includes most of the presumptive signal peptide, whereas the second exon begins at amino acid 16 and ends at amino acid 113. The intron is 101 nucleotides long. Sequences that resemble the heptamer (CACAGTG) and nonamer (ACACAAACC) recognition signals that are involved in the recombination of immunoglobulin¹⁻³ genes and T-cell receptor β -chain genes⁶⁻¹² are next to the 3' end of the second exon, and are separated by 22 nucleotides. The complementary DNA sequence *V_αpY14.1* and the germline sequence *V_αpY14.2* are identical at 251 out of 279 nucleotides (90%) in the protein-coding region, and are identical at 76 out of 93 amino acids (82%). This may not be the gene segment that encodes the pY14 V region.

The results of Southern blotting (see Fig. 1) suggest that there is only one germline C_α gene. To characterize the C region at the molecular level, four λ clones that hybridize with the C region were isolated. Two of these (clones 8 and 18) also hybrid-

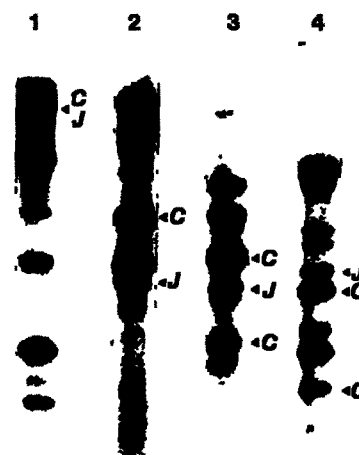


Fig. 1 Southern gel analysis of the V, J and C region genes in human genomic DNA was digested with restriction enzymes, *Eco*RI (lane 1), *Bam*HI (lane 2), *Hind*III (lane 3) and *Bgl*II (lane 4) and analysed by Southern blot^{16,17} using radioactive probes from: (1) the complete pY14 cDNA from Jurkat T-cell line (ref. 16), (2) an oligonucleotide corresponding to the J region sequence (nucleotides 545-593 in Fig. 4 of ref. 16); and (3) the C region probe alone (*Hpa*II/*Hpa*II fragment at nucleotides 591-980 in Fig. 4 of ref. 16). The results using the complete pY14 cDNA probe are shown, but the fragments corresponding to the C and J segments are denoted.

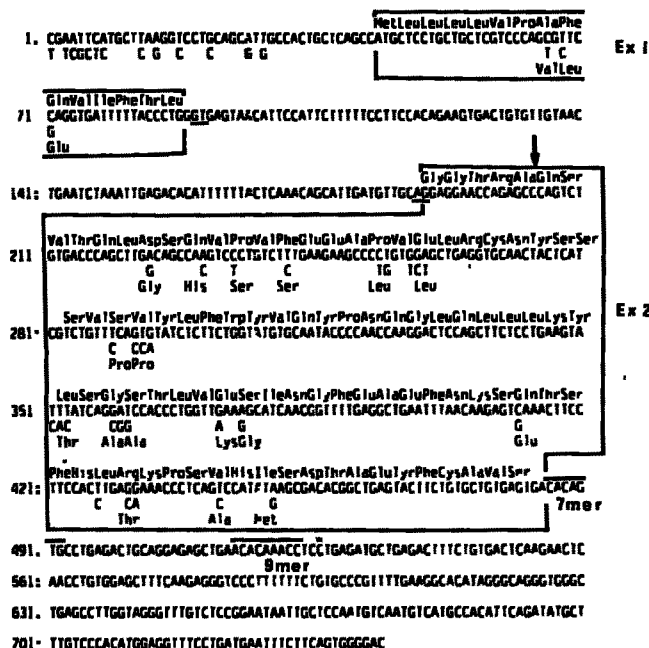


Fig. 2 Nucleotide sequence of a germline V segment *V_αpY14.2* of the human T-cell receptor α -chain genes. Seventeen λ recombinant clones in the human genomic library¹⁸ homologous with the V sequences of pY14 (ref. 16), *Eco*RI/*Sau*3A fragment of pY14 (nucleotides 1-529 in Fig. 4 of ref. 16) were identified by hybridization and washed at 5×SSC, 42°C. One clone, λV13, which contains a 1.3-kb *Eco*RI fragment, hybridized in stringent conditions (0.11×SSC, 68°C). The insert was sequenced using the Sanger dideoxy method²⁴. For the detailed sequencing procedure, see ref. 25. Ex, exon with recombinational signals overlined; underline, splicing signals of AG and GT, arrow, end-of-signal peptide. Differences in nucleotide and deduced amino acids of *V_αpY14.1* from cDNA pY14 (ref. 16) are denoted below the sequences of *V_αpY14.2*.

ized to a J sequence probe of pY14 (*J_αpY14*). Using the technique of 'chromosome walking', λ-clones 21 and 22 were also obtained. A detailed restriction enzyme map of these clones was determined (Fig. 3A). Regions that hybridized with oligonucleotides to α -chain human and murine *J_α* sequences (refs 13-16) are also indicated. The C region is mainly contained on a 5.7-kb

A

Diagram A illustrates the genomic organization of the human $\alpha 1(I)$ procollagen gene. The top line represents the full gene structure, with exons numbered 1 through 28. Introns are indicated by lines with arrows. Below this, the structure of the $\alpha 1$ gene is shown in λ clone 21 and λ clone 22. λ clone 21 contains exons 1 through 17, while λ clone 22 contains exons 1 through 17 and exon 18. A scale bar indicates 1 kb.

B

Diagram B illustrates the structure of the $\alpha 1(I)$ gene in the λ clone 21 and λ clone 22. The top line shows the structure of the $\alpha 1$ gene in λ clone 21, with exons numbered 1 through 28. Below this, the structure of the $\alpha 1$ gene is shown in λ clone 22, with exons numbered 1 through 28. A scale bar indicates 4 kb.

Ex 1

Ex 2

Ex 3

Ex 4

The regions of λ -clones 8, 21 and 22 that hybridize to J_{α} probes are indicated in Fig. 3A. The 3'-most J region that was identified lies close to the 5' end of a 3.6-kb *Bam*HI fragment ~4 kb upstream of the C_{α} gene. This J region hybridizes to a J_{α} pY14 probe under stringent conditions. The sequence of the 3.6-kb *Bam*HI fragment is presented in Fig. 5a. A sequence identical to the J segment of pY14 is found at nucleotides 509–565. Immediately 5' are the heptamer and nonamer recombination signals (CACAGTG and GGTTATCTC, respectively) separated by a 12-nucleotide-long spacer. These sequences are the same as the signal sequences for recombination that are found in immunoglobulin¹⁻³ and the β -chain of the T-cell receptor⁶⁻¹². Examination of the 3.6-kb sequence indicates that two other sequences can be found, including the highly conserved central Phy-Gly-X-Gly sequence. The first of these, from 1,407 to 1,462, is unlikely to be a functional J element because the putative splice donor site is not positioned to maintain the proper reading frame when joined to the C_{α} region. It also has poorly recognizable heptamer and nonamer signal sequences. The second other potential J_{α} segment, from 2,367 to 2,423, matches J_{α} pY14 at 9 of 19 amino acids and has a potential splice donor site at the proper position; however, the heptamer and nonamer sequences do not match the expected sequence very well. An *Alu* repetitive element spans positions 970–1,200 of the 3.6-kb *Bam*HI fragment. Hybridization analysis using oligonucleotide probes to murine and human J sequences indicates that ~12 human J_{α} sequences (Fig. 3A) may be scattered over these three

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A clones (8, 21 and 22). To confirm that at least some of these are *J* sequences, regions denoted *b*, *c*, *d*, *e* and *f* in Figure 3A were also sequenced. Their *J* sequences are illustrated in Fig. 5b-f. It is clear that all these sequences have easily identifiable heptamer and nonamer recombination signal sequences; the highly conserved central Phy-Gly-X-Gly and splice sequences at the 3' end match the concessive sequence well. The sequence illustrated in Fig. 5f is not complete as it is at the end of clone 22. More recent experiments also show that *J_α* sequences extend further 5' to these clones. These *J_α* segments are longer than

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Smooth muscle α -actin is a transformation-sensitive marker for mouse NIH 3T3 and Rat-2 cells

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Heteroploid mouse NIH 3T3 fibroblasts and several rat fibroblast strains (Rat-1, Rat-2 and REF-52) are cell lines of special interest in the field of carcinogenesis because of their extensive use as normal cells in transformation assays for putative cancer-causing genes. Exposure of these cells to carcinogenic chemicals or oncogenic DNA produces anchorage-independent cells with retracted cytoplasm that lack actin cables¹⁻⁷. All human fibroblast strains, normal and transformed, synthesize two electrophoretic forms of actin (β - and γ -actin)⁸⁻¹⁰. In contrast, we discovered that early-passage mouse and rat strains synthesize abundant amounts of each of the three electrophoretic forms of actin (α -, β - and γ -actin) but mouse and rat cancer cells express only β - and γ -actins. We now show that in NIH 3T3 and Rat-2 fibroblasts a third actin, the smooth muscle α isoform, is abundantly co-expressed with β - and γ -actin. In every instance tested following transformation to tumorigenicity, the accumulation of α -actin messenger RNA and α -actin synthesis was greatly inhibited. Shut-down of α -actin expression thus appears to be a reproducible transformation-sensitive marker in rodent fibroblasts.

Figure 1 shows the actin peptides made in untransformed and transformed NIH 3T3 and Rat-2 cells. Using computerized microdensitometry¹¹, we estimate that NIH 3T3 cells synthesize a considerable amount of α -actin, about 25-30% as much as β -actin, and Rat-2 cells synthesize about 25-40% as much α -actin as β -actin. All 68 Rat-2 subclones examined synthesized more α -actin than the parental Rat-2 strain (Fig. 1). The ratio of the rates of α -actin to β -actin synthesis increased from 0.25 to 0.42 as a consequence of subcloning. We and others³ have observed that Rat-2 subclones exhibit flatter, more contact-inhibited monolayers than the parental Rat-2 strain. Thus elevation in the rate of α -actin synthesis is associated with the acquisition of the flatter, more contact-inhibited phenotype on subcloning of Rat-2 cells.

We isolated transformed NIH 3T3 or Rat-2 cell lines from rare foci that arose spontaneously in confluent monolayers. Two such spontaneously transformed cell lines, T-3T3 and FocT, exhibit no α -actin (Fig. 1). We excluded the possibility that these spontaneous transformants arose from a subpopulation of non-transformed cells that do not make α -actin by screening 68 subclonal Rat-2 lines. Each line synthesized α -actin at about the same rate as the Rat-2 subclone shown in Fig. 1. Thus α -actin is a uniformly abundant gene product in Rat-2 cells and in NIH 3T3 cells.

A similar loss of α -actin was detected in all *in vitro*-transformed cell lines that we have examined to date. These additional lines include four separate EJ-Ha-ras oncogene transformed⁴ NIH 3T3 lines, one additional spontaneous transformant of Rat-2, two separate Rat-2 strains transformed with EJ-Ha-ras oncogene, a line transformed by transfection of Rat-2 cells with

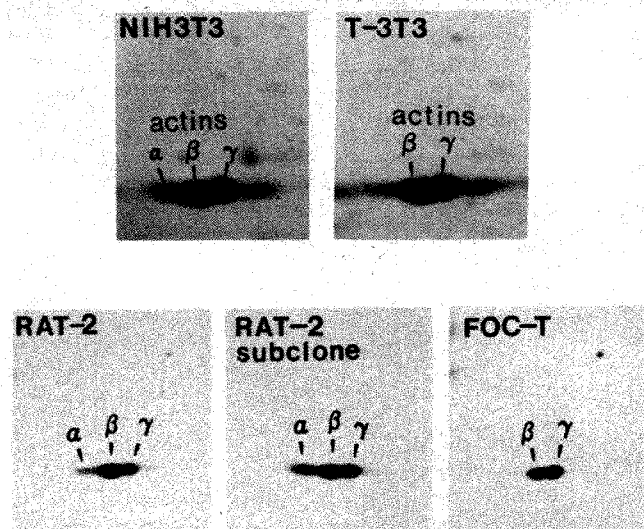


Fig. 1 Autoradiograms of the cellular α -, β - and γ -actin polypeptides of normal and transformed NIH 3T3 and Rat-2 cells separated and resolved by two-dimensional polyacrylamide gel electrophoresis. An ampoule of frozen NIH 3T3 cells² was obtained from Dr Brian Crawford (Los Alamos National Laboratory) who received his 3T3 stock from Dr Esther Chang (Uniformed Services Medical School, Bethesda). The 3T3 stock was cultured through two passages at Los Alamos before preparation of the frozen ampoule used as the seed stock for the present study. The Rat-2 cell line¹ was from Dr Greg Reyes (Stanford University) who obtained his stock from Dr Mike Botchan (University of California, Berkeley). T-3T3 and FocT cell lines were derived from spontaneous foci that appeared in confluent monolayers of NIH 3T3 and Rat-2 cells, respectively. The two focus-derived cell lines were isolated from the focus by adsorption to a 3-mm Whatman filter paper disk which had been soaked in EDTA-trypsin solution (0.25% trypsin, 0.1 mM EDTA). Both cell lines are composed of highly refractile, morphologically transformed cells that produce rapidly growing tumours visible 1 week after subcutaneous inoculation of 2×10^6 cells into nude mice or syngeneic animals. The 'Rat-2 subclone' was a hypoxanthine-aminopterin-thymidine (HAT)-resistant clonal cell line induced by transfection of Rat-2 cells with a cloned thymidine kinase gene (J.L. and R.J., in preparation). All cell cultures were labelled with ³⁵S-methionine for 4 h². Total proteins were solubilized in Lysis A solution^{9,10,24} and the actin polypeptides separated by two-dimensional electrophoresis²⁴. Identification of the actin polypeptides is based on their abundance and relative position in the two-dimensional gel.^{8-10,14,17,18,24,25}

a cloned transforming DNA fragment (*Bam*HI-E) from human herpes simplex virus type 2 (ref. 12 and B. Tanczos *et al.*, in preparation), and four separate transformed cell lines induced by transfection of Rat-2 cells with a combination of cloned transforming DNA fragments (*Xba*I/*Bam*HI fragments EJ and EM) from human cytomegalovirus strain Towne (ref. 13 and R.J. *et al.*, in preparation).

In addition, Franza and Garrels¹⁴ have reported the same finding for rat embryo fibroblasts (REF-52 strain) transfected with the transforming genes of human adenovirus, Kirsten murine sarcoma virus, and simian virus 40. Mouse L-cells, which are highly transformed fibroblasts, also lack α -actin¹⁵.

To confirm α -actin expression in NIH 3T3 and Rat-2 cells, we demonstrated that an α -like actin RNA transcript is present in these cells but is greatly diminished or absent from the transformed cell lines. Figure 2 shows RNA transfer blot hybridization experiments in which total RNA from each cell type has been separated electrophoretically and then hybridized with isotype-specific RNA probes¹⁶ for β -actin (Fig. 2b) or γ -actin (Fig. 2c), or an actin coding sequence probe (Fig. 2a, d). mRNA transcripts for β - or γ -actin are 2,100 base pairs (bp) long^{15,16}. RNA transcripts that code for the three muscle-related α -actins are about 1,600-1,700 bp long¹⁵⁻¹⁸. With the β - and γ -actin-specific probes, only a 2,100-bp transcript was found

Table 1 Modulation of actin mRNA synthesis

		Relative amounts of α -, β - and γ -actin mRNAs			
		Coding probe*	β - and γ -actin isotype-specific†		γ -mRNA modulation‡
		α -mRNA	β -mRNA	γ -mRNA	β -mRNA
		β - plus γ -mRNA			
Mouse (expt 1):					
NIH 3T3	Normal	0.390	1.00	1.00	1.00
T-3T3	Spontaneously transformed§	0.040	2.12	1.16	0.55
Rat (expt 1):					
Rat-2	Normal	0.110	1.00	1.00	1.00
Rat-2 subclone	Normal HAT [¶] , HSV-2 tk gene transfectant	0.080	0.89	0.88	0.99
FocT	Spontaneously transformed§	<0.002	0.86	11.47	13.34
Rat (expt 2):					
Rat-2	Normal	0.094	1.00	1.00	1.00
FocT	Spontaneously transformed§	<0.002	0.68	5.65	8.31
RBET	HSV-2-transformed	<0.002	0.73	2.19	3.00
RXBJMT1	CMV-transformed¶	<0.002	0.84	2.07	2.46
RXBJMT2	CMV-transformed	<0.002	0.74	1.79	2.41
RXBJMT3	CMV-transformed	<0.002	0.69	1.04	1.51
RXBJMT4	CMV-transformed	<0.002	0.93	1.64	1.76

Relative amounts of the actin mRNAs were measured by hybridization with actin-coding or isotype-specific ³²P-DNA probes.

* Relative hybridization of an actin-coding region ³²P-DNA probe (Fig. 2) to either the 2,100-bp β -plus γ -actin mRNA band or the band representing the 1,600–1,700-bp α -actin in RNA in the RNA transfer blots shown in Fig. 2a,d; unsaturated autoradiographic bands resulting from α -specific or β -, γ -specific hybridization were quantified by computerized microdensitometry and the ratio of these measurements is shown.

† Hybridization of β -actin-specific ³²P-DNA probe to the 2,100-bp β -actin mRNA shown in Fig. 2b was quantified by computerized microdensitometry; the amount of hybridization for each total RNA isolate is expressed as a ratio—the amount of hybridization to mRNA from the derivative cell line relative to the amount of hybridization to mRNA from the parental cell line, either NIH 3T3 or Rat-2; or hybridization of the γ -actin-specific ³²P-DNA probe to 2,100-bp γ -actin mRNA as shown in Fig. 2c.

‡ Ratio of γ -actin-specific hybridization (Fig. 2c) to β -actin-specific hybridization (Fig. 2b), or the modulation in amount of γ -actin mRNA accompanying transformation if the amount of β -actin mRNA is kept constant.

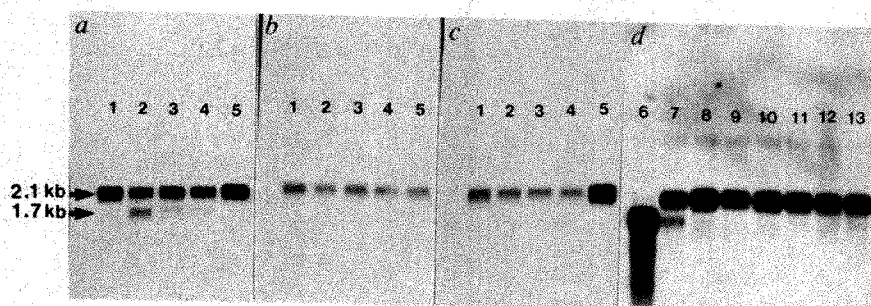
§ Derived from a focus that arose spontaneously in a confluent Rat-2 or NIH 3T3 cell monolayer.

¶ BamHI fragment E (ref. 12).

|| Mixture of XbaI/BamHI fragments EJ and EM¹³.

Fig. 2 Autoradiograms of total RNA transfer blots hybridized with ³²P-DNA probes derived from an actin-coding sequence or isotype-specific 3'-untranslated region (3'UTR) sequences. In a and d, the probe was a PvuII/XbaI DNA fragment from pHM α A-1 (ref. 27) containing the coding region of human skeletal α -actin from amino acid 202 to amino acid 374. This probe hybridizes equally well with muscle and non-muscle actin mRNAs (data not shown). In b, the probe was the human β -actin-specific subclone pHF β A-3'UT¹⁶ and in c the probe was the human γ -actin-specific subclone pHF γ A-3'UT¹⁶. The cell source of total RNA in each electrophoretic lane is as follows: lane 1, T-3T3; lane 2, normal NIH 3T3; lanes 3 and 7, normal Rat-2; lane 4, Rat-2 subclone (Fig. 1); lanes 5 and 8, FocT; lane 6, mouse skeletal muscle^{15,16}; lane 9, RXBJMT1 (Table 1); lane 10, RBET (Table 1); lane 11, RXBJMT2 (Table 1); lane 12, RXBJMT3 (Table 1); and lane 13, RXBJMT4 (Table 1).

Methods. Total RNA (2 μ g) was isolated from each cell line, size-fractionated on 2.2 M formaldehyde-1% agarose gels and blot-transferred to nitrocellulose filters as described previously²⁸. The filters were hybridized with one of three different nick-translated²⁹ DNA probes. Hybridization was done in 4 $\times$ SSC, 50 mM Na₂HPO₄ (pH 7.0), 5 $\times$ Denhardt's³⁰, 10% (w/v) dextran sulphate containing 10⁶ d.p.m. radiolabelled DNA probe per ml at 65 °C. Following hybridization, the filters were washed with five changes of 0.5 $\times$ SSC, 0.1% (w/v) SDS and exposed to Kodak XAR-5 film.



(Fig. 2b, c). With the nonspecific actin-coding probe, an abundant, 1,600–1,700-bp transcript is detected in normal NIH 3T3 and Rat-2 cells, together with the 2,100-bp transcripts for β - and γ -actin (Fig. 2a, d). This result firmly establishes the presence of a third expressed actin mRNA in these cells. The presence of a third actin mRNA also excludes the possibility that the α -actin identified in two-dimensional gels was a post-translationally modified product of β - or γ -actin. Perhaps a trace of α -like transcript is detectable in the transformed NIH 3T3 strain (Fig. 2a, lane 1) but no α -like transcript is found in the transformed Rat-2 strain (Fig. 2a, lane 5). In addition, the herpes simplex virus 2- and four cytomegalovirus-trans-

formed Rat-2 cell lines contain no α -like transcript (Fig. 2d, lanes 9–13; Table 1).

In an attempt to identify which α -actin isoform is expressed in NIH 3T3 and Rat-2 cells, we hybridized replicate RNA blots with 3'-untranslated region probes specific for α (skeletal)- and α (cardiac)-actin mRNA¹⁶; no transcripts were detected (not shown). Thus we conclude by elimination that the α -actin expressed in NIH 3T3 and Rat-2 cells is not β -, γ -, α -skeletal or α -cardiac and is probably the α -actin made in smooth muscle¹⁹. We cannot exclude the formal possibility that the α -actin is an as yet undefined α -actin isotype. However, Vanderkerckhove and Weber²⁰ have demonstrated that cultured

chick embryo fibroblasts synthesize smooth muscle α -actin in addition to non-muscle β - and γ -actin. This prior observation strongly supports the conclusion that the α -actin we detect in rodent fibroblast cell lines is also of the smooth muscle type.

In the spontaneously transformed Rat-2 cell line FocT there was an additional unexpected change in actin expression. Rat-2 cells accumulate less γ -actin than β -actin (Fig. 1), so that the ratio of γ -actin to β -actin is 0.4. The spontaneously transformed Rat-2 cell line accumulates more γ -actin than β -actin, amounting to a threefold up-regulation of γ -actin relative to β -actin (Fig. 1). The amount of γ -actin mRNA is up-regulated even more dramatically relative to the β -actin mRNA (Fig. 2b, c, lane 5). Assuming that these changes are not related to altered mRNA stability, we estimate that the rate of γ -actin gene transcription has increased 8-13-fold in this transformed strain (Table 1).

To examine the relative significance of the actin protein modulations, we have analysed the 500 most abundant polypeptide species in high-resolution two-dimensional gels of total proteins from most of the normal and transformed Rat-2 cells discussed in the present paper. We consistently find no qualitative changes in polypeptide synthesis by Rat-2 cells other than the loss of α -actin. The same finding is implied by Franza and Garrels¹⁴, although they and Matsumura *et al.*²¹ also report that the microfilament-associated tropomyosin subunits of REF-52 are modulated quantitatively but variably among different types of transformed cells. Thus, at least among the 500 abundant cellular proteins of rat fibroblasts, the alterations of actin expression are not a general phenomenon shared by other proteins.

Rat-2 and NIH 3T3 cells are similar in their shape and relatively flat morphology¹, they are both aneuploid^{1,2}, and will consistently form subcutaneous tumours in syngeneic animals, but only after long latent periods of 10-12 weeks (J.L. and R.J., unpublished results). By contrast, all of the transformed cell lines mentioned in this study produce, within 1-3 weeks, solid tumours that grow rapidly to a mass 4 cm in diameter by 4-5 weeks. Morphologically these transformed cells are typical of other transformed cells^{3-8,12-14,22} but contrast with untransformed Rat-2 and NIH 3T3 cells in that their cytoplasm is retracted and refractile on the plastic substratum. Thus modulations in actin expression and abundance of the types described in this study are associated with microfilament rearrangement and subsequent alterations in cell shape, leading to elevation of tumorigenic potential. Indeed, one acute transforming oncogene of the Gardner-Rasheed feline sarcoma virus encodes the N-terminal amino-acid sequence (130 residues) of actin²³. This finding supports the hypothesis that alteration of the microfilament system through qualitative changes in actin expression^{8-10,14,22-26} or organization^{7,8,26} may be an important aspect of the neoplastic transformation process.

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Detection of deletions spanning the Duchenne muscular dystrophy locus using a tightly linked DNA segment

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The Duchenne muscular dystrophy (DMD) locus has been localized to the short arm of the human X chromosome (Xp21) by detection of structural abnormalities¹⁻⁵ and by genetic linkage studies⁶⁻⁸. A library highly enriched for human DNA from Xp21 was constructed⁹ using DNA isolated from a male patient who had a visible deletion and three X-linked disorders (DMD, retinitis pigmentosa and chronic granulomatous disease)⁵. Seven cloned DNA probes from this library and the probe 754 (refs 5, 8) are used in the present study to screen for deletions in the DNA isolated from 57 unrelated males with DMD. Five of these DMD males are shown to exhibit deletions for one of the cloned DNA segments and at least 38 kb of surrounding DNA. In addition, two subclones from the same region detect four restriction fragment length polymorphisms which exhibit no obligate recombination with DMD in 34 meiotic events. These new DNA segments will complement the existing Xp21 probes⁴⁻⁹ for use in carrier detection and prenatal diagnosis of DMD. Elucidation of the end points of the five deletions will help delineate the extent of the DMD locus and ultimately lead to an understanding of the specific sequences involved in DMD.

The DNA of 57 unrelated DMD males was tested for deletions of the following 8 cloned DNA segments absent from the male patient with a visible deletion⁵: pERT55-2, pERT84, pERT87, pERT145, pERT378, pERT379, pERT469 and 754 (pERT, plasmids isolated from a library enriched for Xp21 DNA segments⁹). Based on a previously described strategy, 8 probes from a small region of the genome should increase the probability that one probe will detect a small deletion at a specific locus in the region¹⁰. Among the 8 clones tested, only one, pERT87, was found to be absent from the DNA of 5 of the 57 unrelated DMD males.

pERT87 was screened by hybridization¹¹ against total human genomic libraries constructed in Charon 35 (ref. 12) and EMBL-3 (ref. 13) to obtain more surrounding DNA. After chromosomal

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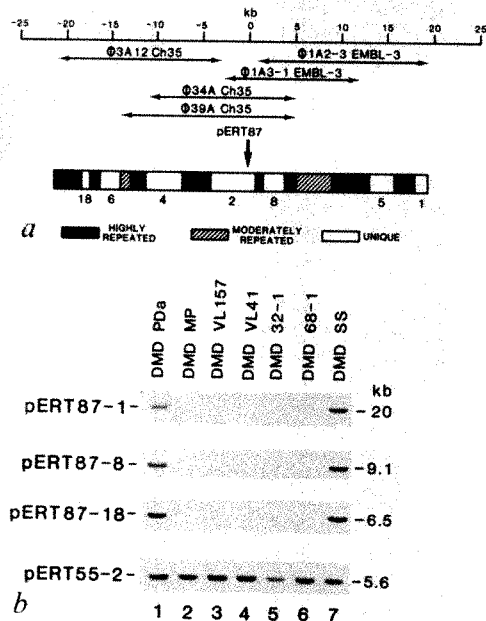


Fig. 1 **a**, Schematic map of 41 kilobases of genomic DNA around the original 200 base pairs, pERT87 (0 on kb scale). The subcloned segments that were free of human repeated sequence elements are indicated numerically below the block diagram, and the size of the segment is represented by an open block. Segments containing highly repeated sequences are indicated as darkened areas, and those containing moderately repeated segments as cross-hatched areas. **b**, Southern blot¹⁴ analysis of DNA from seven males with DMD. Lanes 1-7 contain *Pst*I-digested DNA of the patients. Two identical nitrocellulose filters were hybridized with pERT87-18 and pERT87-1. The same filters were then rehybridized with pERT87-8 and pERT55-2, respectively. The autoradiographs are presented as separate segments of each original autoradiograph. The fragment hybridized is indicated to the left of the figure; the sizes of hybridizing fragments are indicated on the right and were calculated from ³²P-end-labelled *Hind*III-cleaved λ DNA. The numerical designation of the subclone used as a probe and the probe's relative position in 'walked' DNA are indicated in **a**. The designation above each lane is the nomenclature for each DMD male.

Methods. Five overlapping bacteriophage clones (lines with arrows below kb scale) were isolated¹¹ from two total human DNA recombinant libraries constructed in Charon 35 (ref. 12) and EMBL-3 (ref. 13) (generously provided by Drs S. Orkin and Y. Shiloh, respectively). Each library was constructed from size-selected (13-21 kb) partially *Mbo*I-digested human DNA and the respective phage vector. Screening¹¹ was against nitrocellulose filters lifted from primary plates (the libraries were never amplified). Hybridizing phage were plaque-purified and *Hind*III-digested human DNA segments were subcloned²² into *Hind*III-cleaved and dephosphorylated pBR322 (New England Biolabs). The subclones were digested with various restriction enzymes (*Eco*RI, *Xba*I and *Kpn*I) to yield smaller fragments free of human repetitive sequence elements. Genomic DNA was isolated from cell nuclei of whole blood leukocytes as described previously⁷ and digested with *Pst*I in buffers recommended by the manufacturer. The digested DNA (1.5 μ g) was separated by electrophoresis on a horizontal 0.7% agarose gel and transferred to nitrocellulose¹⁴. The unique-sequence subclones pERT87-18, pERT87-8, pERT87-1 and pERT55-2 were ³²P-radiolabelled using T4 DNA polymerase and radiolabelled fragments were recovered from low melting point agarose gels⁷. Prehybridization of nitrocellulose filters was undertaken as described previously⁷ and hybridization was at 45 °C in 50% deionized formamide, 4 $\times$ SSC (1 $\times$ SSC = 0.15 M NaCl, 0.015 M Na citrate), 0.05 M sodium phosphate buffer (pH 6.8), 8% dextran sulphate, 10 $\times$ Denhardt's, 100 μ g ml⁻¹ transfer RNA, 25 μ g ml⁻¹ sheared salmon sperm DNA, 0.1% SDS, 1 mM EDTA and 10⁶ c.p.m. ml⁻¹ of radiolabelled DNA. Filters were washed in 0.1% SDS and 0.1 $\times$ SSC several times at room temperature and then for 1 h at 55 °C. Autoradiography was at -70 °C with a DuPont intensifying screen for 1-5 days.

'walks' in both directions from the original pERT87 clone, five bacteriophage clones were isolated which had a combined insert size of 41 kilobases (kb) (Fig. 1a). Small unique-sequence subclones from the recovered pERT87 human inserts were hybridized¹⁴ to *Pst*I- or *Taq*I-cleaved DNA of the 57 DMD males. The results for the five deletions are presented in Fig. 1b together with results from two DMD boys who do not bear deletions of the pERT87 region. The subclones pERT87-18, pERT87-8 and pERT87-1 are absent from the DNA of these five unrelated individuals (Fig. 1b, lanes 2-6). These three subclones span a region of 38 kb of genomic sequences (see schematic map, Fig.

Table 1 Detection of deletions in DMD males

	Total	Deletions
DMD X chromosomes		
Familial origin	17	3 (18%)
Sporadic origin	40	2 (5%)
Total	57	5 (9%)
Normal X chromosomes	61	0

The DNA isolated from 57 DMD males, 47 normal males and 7 normal females was cleaved with *Pst*I or *Taq*I. Diagnosis of DMD was established by characteristic history, physical examination, increased CPK levels and a positive muscle biopsy²¹. None of the five boys with deletions exhibited mental retardation, developmental delay, recurrent infections or retinal abnormalities. Following separation of digested DNA by electrophoresis, the DNA samples were transferred to nitrocellulose and hybridized with pERT87 phage subclones or other Xp21 cloned DNA fragments as described in Fig. 1 legend. All 57 DMD X chromosomes were tested for deletion of the pERT87 region, as were all 61 normal X chromosomes. In some cases only 37 DMD males were tested for deletion of other pERT clones or 754, but all 5 boys deleted for the pERT87 region were tested for deletion at other pERT and 754 loci. Only the pERT87 region was found to be deleted in any of the 57 DMD males. Deletions were defined as complete absence of hybridization of a cloned fragment to any restriction fragment in a particular endonuclease digest. In all cases at least one other pERT clone or 754 was shown to be present on the same nitrocellulose filter on which the pERT87 region exhibited deletion. Familial cases of DMD were determined either by extensive family history of DMD or, in a few cases, by elevated CPK^{15,16} in mothers of DMD males. Sporadic cases were those where no elevated CPK was observed in a mother with no family history of the disease. Normal individuals were defined as fathers of males with DMD or other X-linked diseases, random normal individuals or, in some cases, individuals affected by other X-linked diseases.

1a) and therefore give a lower limit of 38 kb to all five deletions. If the deletion in the male patient⁵ lacking all eight DNA fragments is 2,000-5,000 kb and if the eight cloned fragments are uniformly distributed within the deleted region in normal DNA, then an upper estimate of the five deletions detected by pERT87 is 250-600 kb.

The five deletions detected represent 9% of DMD males tested. Of 61 normal X chromosomes (47 males and 7 females), no deletions were found in the pERT87 region (Table 1). Among the boys who were found to have deletions, two were sporadic cases of DMD with no previous family history. The other three were familial cases of DMD (determined by elevated creatine phosphokinase (CPK) levels in suspected carrier females^{15,16} or a family history of DMD). The results given in Table 1 and Fig. 1b are consistent with results obtained with two other X-linked disorders, Lesch-Nyhan syndrome¹⁷ and ornithine transcarbamylase (OTC) deficiency¹⁸, in which 18 and 6.6% of patients were found to have partial or complete deletions of genomic DNA sequences complementary to the cloned cDNAs for hypoxanthine-guanine phosphoribosyltransferase and OTC, respectively.

Several unique subclones from the pERT87 region were hybridized to nitrocellulose filters containing immobilized DNA samples isolated from four unrelated females cleaved with 24 different restriction enzymes⁷. The subcloned probe pERT87-8 (a 1.3-kb *Xba*I fragment next to the original pERT probe, pERT87; see Fig. 1a) was found to recognize both a *Bst*XI and a *Taq*I restriction fragment length polymorphism (RFLP). Figure 2a shows the segregation of the *Bst*XI RFLP and demonstrates mendelian inheritance with no obligate recombinants in five meiotic events. The segregation of the *Taq*I RFLP (Fig. 2) shows no obligate recombination in six meiotic events. To date, the 2 RFLPs seen by pERT87-8 have not recombined in 34 meiotic events in 10 DMD families. A lod score was calculated¹⁹ based on segregation results of the two pERT87-8 RFLPs in these 10 DMD families (two presented here); there were seven meioses in which the phase was known and 24 in which it was not known. The only female individuals who were judged to be carriers or normal on the basis of CPK levels are those presented

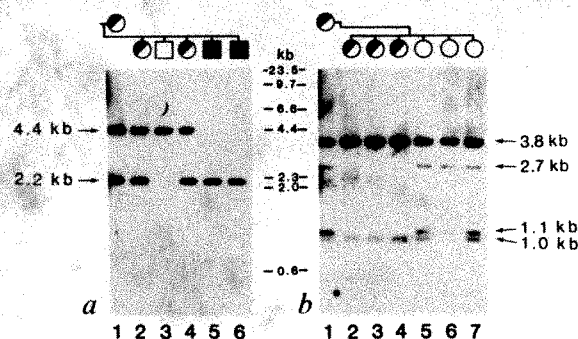


Fig. 2 Segregation of pERT87-8 in DMD families. **a**, DNA was isolated from individuals within a family in which the DMD trait segregated (indicated by darkened symbols), and the DNA was cleaved with *Bst*XI. Electrophoresis, blotting and hybridization were as described for Fig. 1b. On *Bst*XI digestion of genomic DNA, pERT87-8 hybridizes to 4.4- and 2.2-kb *Bst*XI fragments in females heterozygous for the RFLP. The 4.4-kb fragment is observed in 60% of X chromosomes and the 2.2-kb fragment in 40% of X chromosomes (28X chromosomes tested). The carrier mother exhibits the heterozygous pattern of the 4.4/2.2-kb *Bst*XI alleles, as do her two carrier daughters (lanes 2, 4). The lower allele (2.2 kb) is present in both affected DMD probands (lanes 5, 6) while the upper allele (4.4 kb) is present in the unaffected son (lane 3). The father in this family has a 4.4-kb *Bst*XI hybridizing fragment (data not shown). The female individuals in lanes 2 and 4 were suspected of being carriers of the DMD trait based on elevated CPK values^{15,16,21}. Fragment sizes were calculated by comparison with ³²P-end-labelled *Hind*III-cleaved λ phage DNA (shown in centre of figure). **b**, The DNA isolated from individuals of a family segregating the DMD trait was cleaved with *Taq*I. The pERT87-8 probe hybridizes to a 3.8-kb, 2.7-kb, 1.1-kb and 1.0-kb fragment in females heterozygous for the RFLP. The 1.0-kb *Taq*I fragment is present in all individuals, whereas the RFLP is revealed by the presence or absence of a *Taq*I site on a 3.8-kb fragment. The rarer allele is a 3.8-kb fragment (27% of X chromosomes), and the common allele is represented by 2.7- and 1.1-kb fragments (73% of X chromosomes tested). The carrier mother (lane 1) exhibits the heterozygous *Taq*I pattern of the 1.1-, 2.7/3.8-kb bands. Her three DMD carrier daughters (lanes 2-4), all of whom have affected sons, show two-copy hybridization to the large 3.8-kb allele, while the three suspected non-carrier daughters (based on their CPK values; lanes 5-7) exhibit the heterozygous pattern. This demonstrates that the X chromosome with the 3.8-kb allele from the carrier mother is inherited by the three carrier daughters along with the 3.8-kb allele from the father. The affected sons of these carrier women all inherited a 3.8-kb and a 1.0-kb *Taq*I fragment from their mothers (data not shown). Females were determined to be carriers of the DMD trait by elevated CPK and sons with DMD; non-carrier women were determined by normal CPK. The dark smudges between 2.7 and 1.1 kb in lanes 1-5 are nonspecific and are caused by the need to autoradiograph the nitrocellulose filter for 6 days to visualize the faintly hybridizing 2.7-, 1.1- and 1.0-kb fragments. These only occurred in this autoradiograph.

in Fig. 2; most results were obtained with affected or normal sons of obligate heterozygous DMD females. The coincidence of deletions for pERT87 and the DMD trait was not used in the calculation of lod scores. The calculated maximum lod score was 7.6 at $\theta = 0$ centimorgans at confidence limits, set by lod scores one unit lower, of 0 to 7 centimorgans. A more precise recombination fraction will take many more informative meioses.

To characterize more precisely the sporadic or familial nature of the deletions detected by the pERT87 probes, the *Bst*XI RFLP was used to tag specific X chromosomes in two families containing DMD boys bearing deletions. In the first family (sporadic), the mother (Fig. 3a, lane 5) of a DMD boy with a deletion (Fig. 3a, lane 4) is heterozygous for the *Bst*XI RFLP. This woman must have two copies of the pERT87-8 locus and is not heterozygous for the deletion. In the second family (familial), the grandmother (Fig. 3b, lane 1) is either hemizygous or homozygous for the 2.2-kb *Bst*XI allele, but her daughter (Fig. 3b, lane 2) has only the 4.4-kb allele, presumably inherited from her father. As the daughter has no hybridizing 2.2-kb allele which she should have inherited from her mother and as the hybridization intensity of the 4.4-kb allele is similar to that of a single X chromosome in the control female (Fig. 3b, lane 5), we presume that the daughter is heterozygous for the deletion evident in her two DMD sons (Fig. 3b, lanes 3, 4).

The *Bst*XI and *Taq*I RFLPs are predicted to be heterozygous in 48 and 39%, respectively, of women tested (allele frequencies

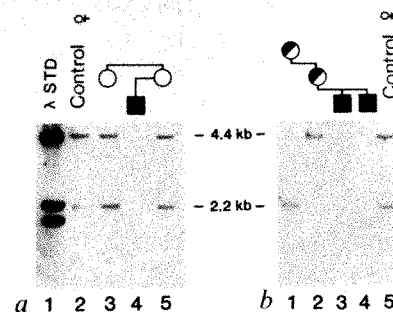


Fig. 3 Analysis of deletions for pERT87-8 in DMD families. **a**, DNA was isolated from a mother of a DMD boy with a deletion and from her sister. Electrophoresis of *Bst*XI-cleaved DNA and hybridization of pERT87-8 were as described in Fig. 1 legend. The open circles above lanes 3 and 5 indicate the two sisters and the darkened symbol above lane 4 represents the DMD son. An unrelated female heterozygous for the *Bst*XI RFLP is presented in lane 2. The sizes of hybridizing fragments given in the centre were calculated from a ³²P-end-labelled *Hind*III digest of λ DNA (lane 1). **b**, DNA isolated from the grandmother (lane 1), mother (lane 2) and two DMD sons (lanes 3, 4) was cleaved with *Bst*XI and the DNA hybridized with pERT87-8 as described in Fig. 1 legend. DNA isolated from an unrelated female heterozygous for the *Bst*XI RFLP is included in lane 5. The darkened symbols above the lanes indicate the DMD trait.

given in Fig. 2 legend). After identifying the pERT87-8 RFLPs, two additional RFLPs were found to be recognized by pERT87-1 (a 1.4-kb *Kpn*I/*Sal*I fragment 16 kb away from pERT87-8; Fig. 1a). Allele frequencies of these RFLP alleles have been determined and mendelian inheritance established. On *Xmn*I digestion, pERT87-1 detects 8.7- and 7.5-kb fragments in heterozygous females (allele frequencies 0.72 and 0.28, respectively). With *Bst*NI digestion, pERT87-1 detects fragments of 3.1 and 2.45 kb/0.65-kb fragments with an invariant 0.7-kb fragment in heterozygous families (allele frequencies 0.52 and 0.48, respectively). One or other of the pERT87-1 RFLP alleles was found to be useful in females from whom no useful information could be obtained using the pERT87-8 *Bst*XI or *Taq*I RFLPs. If there is no linkage disequilibrium of these four RFLPs at the pERT87 region, the expected combined heterozygosity is 0.90. The observed heterozygosity in women studied with all four RFLPs is slightly greater than 0.6. The pERT87-8 region has been mapped⁹ distal to the (X; autosome) translocation breaks within Xp21 in DMD females^{2,3} and should complement the existing proximal probe 754⁵ for use in carrier detection and prenatal diagnosis of DMD. The ability of pERT87-8 and pERT87-1 to distinguish tightly linked RFLP alleles in women at risk for DMD and detection of presumed deletions at the locus make these the most informative probes to date in following the DMD X chromosome.

The deletions detected by pERT87 subclones in the five unrelated DMD males provide a unique tool for defining the extent of the DMD locus. At present, all five individuals lack a minimum of 38 kb of genomic sequences. Expansion of the cloned region by chromosomal 'walking' on total genomic or sorted human X-chromosome bacteriophage libraries²⁰ should elucidate the extent of these deletions. Newly acquired unique-sequence DNA segments can be used to screen for additional deletions, structural rearrangements or new RFLPs. Determination of the common region deleted or rearranged in DMD males should define the exact location of the DNA sequences involved in DMD. Analysis of the cloned DNA from this region should lead to an understanding of the molecular mechanisms that give rise to the phenotype of DMD.

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Relationship between an enhancer element in the human antithrombin III gene and an immunoglobulin light-chain gene enhancer

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Enhancers, *cis*-acting transcriptional control elements have been described in both viral and cellular genes¹. They influence transcription in a quantitative fashion, act over relatively large distances (several kilobases, kb) and behave independently of their position and orientation. Enhancers have been described in immunoglobulin, chymotrypsin and insulin genes²⁻⁹. They bear little homology with each other except for an 8-base pair (bp) 'consensus' core element, GTGG^{AAA}_{TTT}G (refs 10, 11), but even this element is sometimes non-homologous⁹. I have searched for such elements in the human antithrombin III (AT-III) gene. AT-III is an important coagulation protein which inactivates thrombin¹². It is produced by the liver and, to a lesser extent, by the kidney¹³. Here, I report that the 5' flanking region of the AT-III gene encodes a segment homologous with the enhancer containing the joining-constant_κ (*J_κ-C_κ*) intron of immunoglobulin *κ*-chain genes. This extensive homology suggests the existence of regulatory factors that recognize common DNA sequences in lymphoid tissues and in those which express AT-III.

Two regions in the 5'-flanking region of the AT-III gene demonstrate sequence homology with sites in the *J_κ-C_κ* introns of murine or human immunoglobulin *C_κ* genes (Fig. 1). Region 1 corresponds to nucleotides 252-264 of the AT-III gene¹⁴ and to nucleotides 3,904-3,916 of the *J_κ-C_κ* intron of human and murine *C* genes^{15,16}. Ten of 13 residues (77%) were homologous in the AT-III and human *J_κ-C_κ* sequences and 9 of 13 residues (69%) were homologous in the AT-III and murine *J_κ-C_κ* sequence. A second, more extensive, region of homology (17 of 22 bases, 77%) was observed between nucleotides 291 and 312

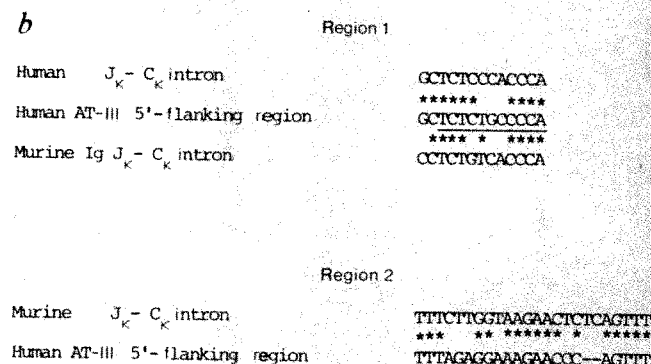
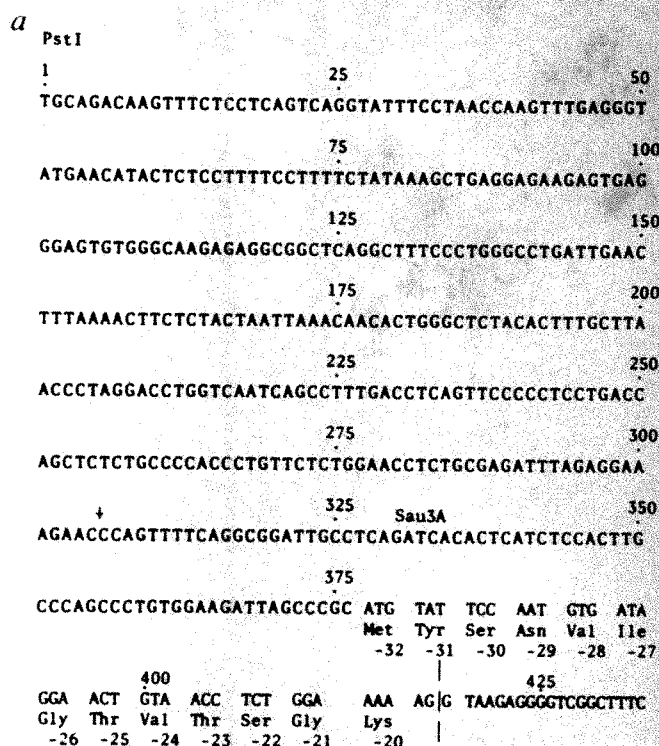


Fig. 1 Homologies between the human AT-III 5'-flanking region and human and mouse *J_κ-C_κ* large introns. **a**, DNA sequence of the immediate 5' end of the AT-III gene. The sequence shown here is a portion of a 1.2-kb *Pst*I genomic subclone (pAT1.2) containing 304 bp upstream flanking region, 72 bp 5'-untranslated region and codons for the first 13 amino acids of the signal peptide¹⁴. The rest of the subclone contains ~800 bp of intervening sequence. The arrow at position 304 denotes the transcriptional start site. **b**, Sequence homologies between AT-III and *J_κ-C_κ* introns. In region 1, the AT-III sequence shown is that between nucleotides 252 and 264. The *J_κ-C_κ* sequence is that between nucleotides 291 and 312, according to the numbering system of Max *et al.*¹⁶. In region 2, the AT-III sequence is that between nucleotides 291 and 312 and the *J_κ-C_κ* sequence is that between nucleotides 3,868 and 3,892 (refs 15, 16). Underlined regions correspond to polyoma virus or core enhancer sequence homologies¹⁷⁻¹⁹.

of AT-III and nucleotides 3,869-3,892 of the murine *J_κ-C_κ* region, although homology with the human *J_κ-C_κ* region was not observed. Although regions 1 and 2 are short and homology could conceivably be fortuitous, it was striking that both regions bear similarities to known viral enhancer elements. For example, in region 1, the underlined area (Fig. 1b) is homologous with a portion of the polyoma virus enhancer TCTCCACCA¹⁷⁻¹⁹. Similarly, the underlined area in region 2 is homologous with the 'core' consensus sequence GTGG^{AAA}_{TTT}G, thought to be^{10,11} common to some eukaryotic viral enhancer elements. Thus, the 5'-flanking region of the human AT-III gene might contain functional enhancer activity; I tested this suggestion in an *in vivo* transfection assay using the chloramphenicol

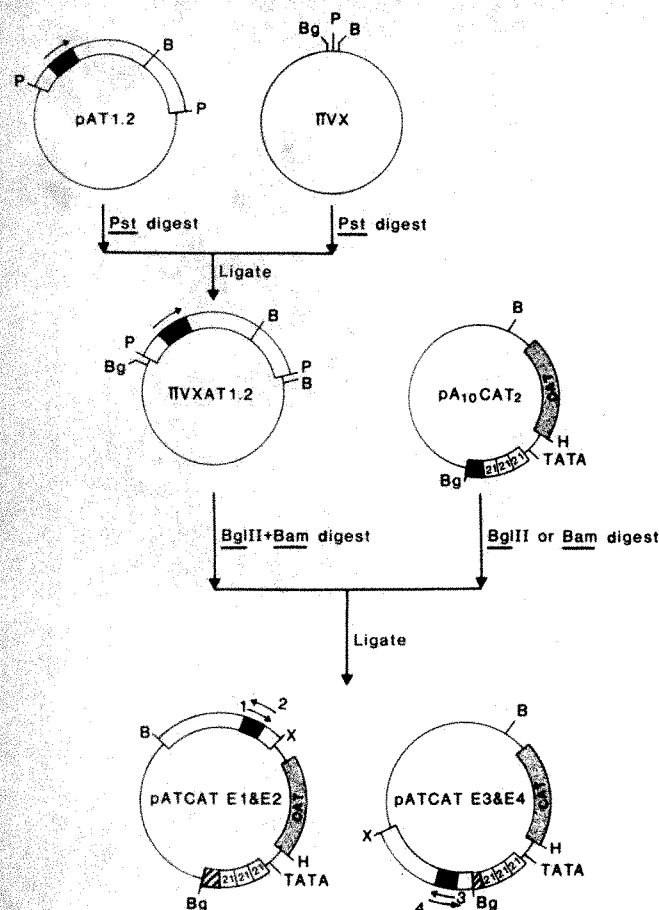


Fig. 2 Construction of AT-III CAT vectors. pAT1.2 is the 1.2-kb AT-III genomic subclone described in Fig. 1 legend. The insert was excised with *Pst*I and ligated into the miniplasmid π VX³⁷ in such an orientation that the region containing the enhancer element was bounded by a 5' *Bgl*II site in the π VX polylinker and the 3' *Bam*HI site in IVS-1 of the AT-III gene. Following digestion of π VXAT1.2 with *Bgl*II and *Bam*HI, the 800-bp-long enhancer-containing fragment was ligated in both possible orientations into the *Bgl*II (upstream) or *Bam*HI (downstream) sites of the enhancerless CAT expression vector pA₁₀CAT₂. The resulting four constructs were designated pATCAT-E1-E4, with only orientations 1 and 3 shown here for simplicity. The arrows indicate the 5'→3' direction of the enhancer element in each of the constructs. Note that the relative proximities of the enhancer element to the CAT promoter are therefore E3>E4>E2>E1. P, *Pst*I; B, *Bam*HI; Bg, *Bgl*II; H, *Hind*III; X, *Bam*HI or *Bgl*II sites destroyed by ligation of nonregenerative GATC sticky ends. Before transfection studies, all plasmids were isolated by the alkaline lysis method³⁸ and doubly banded in CsCl to ensure complete removal of contaminating nucleic acids. Open box, AT-III-derived sequences; solid box, *J_κ-C_κ* enhancer-like region; solid lines, plasmid-derived sequences; stippled box, CAT gene; cross-hatched box; truncated SV40 enhancer region; 21, SV40 21-bp repeats.

acetyltransferase (CAT) assay²⁰. Restriction fragments containing the putative enhancer element from the AT-III gene were cloned into the enhancerless vector pA₁₀CAT₂ (Fig. 2) and their effect on transcription quantitated by examining cell extracts for the presence of CAT following transfection. A positive control plasmid, pSV₂CAT, contains a simian virus 40 (SV40) enhancer and directs the synthesis of large amounts of CAT. Each plasmid was transfected into four different eukaryotic cell lines, two of which, the COS-1 African green monkey kidney line²¹ and the Alexander human hepatoma line²² are derived from tissues that normally express AT-III (ref. 13). Cell extracts were prepared and assayed for CAT activity 48 h later²⁰. CAT activities were normalized to the results of Hirt extracts²³ to allow for variations in the transfected DNA uptake and replication. The results (Fig. 3) can be summarized as follows: (1) CAT activity was significantly enhanced above background by the

presence of 800 bp of AT-III gene sequence containing the *J_κ-C_κ* homologies; (2) the level of enhancement was generally in inverse proportion to the distance of the putative enhancer element from the SV40 early promoter, although some variability was seen between experiments; (3) enhancer activity was independent of the orientation of the AT-III sequences; (4) enhancer activity was seen only in those cells derived from tissues that naturally produce AT-III, that is, COS-1 (kidney) and Alexander hepatoma (liver). Examination of Hirt extracts of transfected cells showed only small differences in plasmid copy number within any single experiment, indicating that the increased CAT activity was not the result of preferential replication of enhancer-containing constructs in COS-1 cells or of variability in DNA uptake (my unpublished data). Therefore, I conclude that the 5' end of the human AT-III gene contains sequences that potentiate heterologous gene transcription in a tissue-specific manner when assayed by transient expression.

The extensive sequence homology observed between AT-III and immunoglobulin *J_κ-C_κ* enhancers suggested that there is also functional homology. This possibility was tested by introducing a murine immunoglobulin *J_κ-C_κ* enhancer into Alexander and COS-1 cells and the AT-III enhancer into a murine myeloma line and measuring CAT activity 48 h later. In the former case, the immunoglobulin *J_κ-C_κ* enhancer element was cloned into the *Bgl*II site of pA₁₀CAT₂. The resultant plasmid, designated pA₁₀CAT₂- κ , was then introduced into Alexander or COS-1 cells by calcium phosphate precipitation. The plasmids pA₁₀CAT₂- κ and pATCAT-E3 were transfected into 10⁷ M12 murine myeloma cells using the electroporation technique²⁴ and cell extracts were assayed for CAT after 48 h (Table 1). As observed previously, the AT-III enhancer functioned in both Alexander and COS-1 cells. In a similar fashion, pA₁₀CAT₂- κ also functioned above background when introduced into M12 cells. More significant, however, was the observation that the AT-III enhancer functioned in M12 cells and that the immunoglobulin *J_κ-C_κ* enhancer functioned in at least one of the cell lines (COS-1) derived from AT-III-producing tissues. CAT activity was generally less in the heterologous cells: the AT-III enhancer functioned better in COS-1 and Alexander cells whereas the *J_κ-C_κ* enhancer functioned better in M12 cells. pA₁₀CAT₂- κ functioned at only

Table 1 Relative activity of AT-III and *J_κ-C_κ* enhancers in myeloma cells, hepatocytes and COS-1 cells

Transfected plasmid	M12 myeloma	CAT activity in	
		Alexander hepatoma	COS-1
pATCAT-E3	4.0	4.3	12.8
pA ₁₀ CAT ₂	7.2	1.5	4.4
pA ₁₀ CAT ₂ - κ	1.0	1.0	1.0

Plasmid pATCAT-E3 is described in Fig. 2 legend. pA₁₀CAT₂- κ was constructed by removal of the 1.25-kb *Hind*III/*Hpa*I fragment from the rearranged murine gene MOPC-41¹⁶. The fragment was repaired with DNA polymerase (Klenow fragment) and deoxynucleoside triphosphates. *Bgl*II linkers were added, followed by ligation into the *Bgl*II site of pA₁₀CAT₂. The orientation was such that the former *Hind*III site was brought closer to the SV40 early promoter. 20 μ g of each plasmid was introduced into either Alexander or COS-1 cells by standard calcium phosphate DNA co-precipitation onto 5 $\times$ 10⁵ cells plates the day before. For M12 cells, transfection was accomplished by electroporation of 10⁷ myeloma cells in 0.5 ml phosphate-buffered saline (PBS). The procedure was essentially as described previously²⁴ using an Isco Model 494 electrophoresis power supply. Voltage was adjusted to 2,000 V, current to 0.9 mA and the adjustable current and wattage set at a minimum. Cells were kept at 0 °C in PBS during electroporation and for 10 min afterwards before being replated in modified Eagle's medium (NEM)/10% fetal calf serum (FCS). Following transfection, each cell line was grown for 48 h in MEM/FCS, at which time CAT activity was measured in extracts. The values shown represent the increase over the amount of CAT conversion seen after transfection with pA₁₀CAT₂ alone (value arbitrarily set at 1).

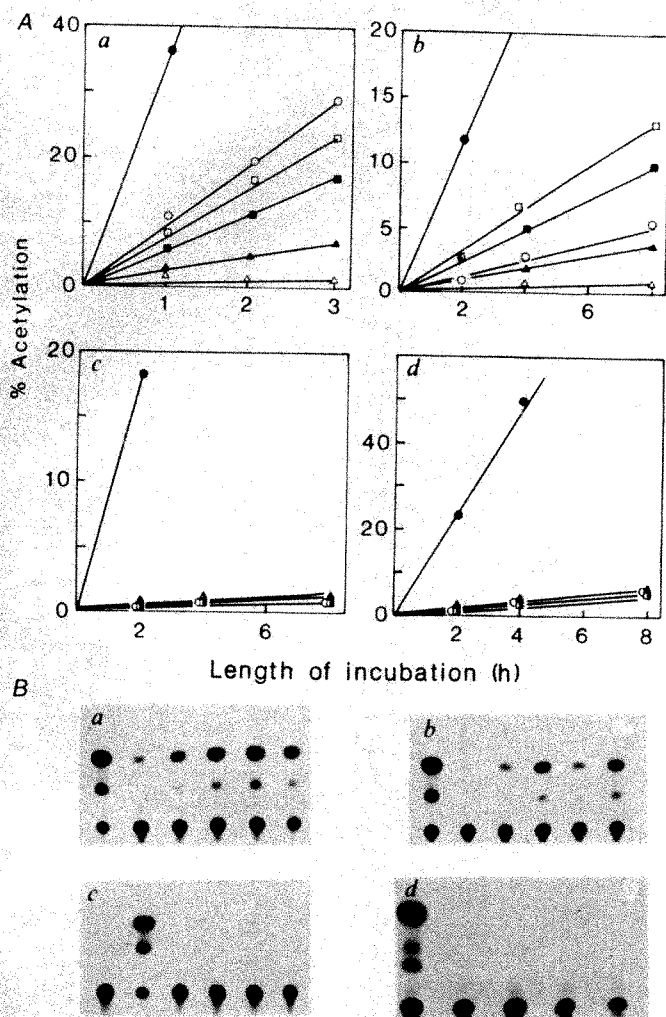


Fig. 3 Tissue-specific CAT expression. **A**, Kinetics of CAT activity in four different cell lines: **a**, COS-1; **b**, Alexander human hepatoma; **c**, HeLa; **d**, mouse thymidine kinase-negative L cells. Transfections were with pSV2CAT (●); pA₁₀CAT₂ (△); pATCAT-E1 (▲); pATCAT-E2 (■); pATCAT-E3 (○); pATCAT-E4 (□). All points in this and subsequent experiments are normalized for differences in plasmid copy number as determined by DNA dot-blots of Hirt extracts²³. **B**, Thin-layer chromatograms of CAT conversion products. Spots nearest the bottom represent unacetylated ¹⁴C-chloramphenicol. **a**, COS-1; **b**, Alexander; **c**, HeLa; **d**, L cells. From left to right in each lane, the products from transfections with pSV2CAT, pA₁₀CAT₂, pATCAT-E1, pATCAT-E2, pATCAT-E3 and pATCAT-E4, respectively, are spotted. In **c** the pA₁₀CAT₂ and pSV2CAT lanes have been reversed. Each set of experiments was performed at least twice with three different plasmid preparations.

Methods. All cells were grown in Dulbecco's modified Eagle's medium (MEM) supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 µg ml⁻¹ streptomycin and 100 U ml⁻¹ penicillin G. Cells were split into 100-mm tissue culture plates at 5×10^5 cells per plate and 24–48 h later, 20 µg of each of the four AT-III CAT constructs shown in Fig. 2 were introduced into the cells by calcium phosphate precipitation³⁹. As negative and positive controls, pA₁₀CAT₂ or the SV40 enhancer-containing plasmid pSV2CAT were also used²⁰. Precipitates were kept on the cells for 5 h, after which the cells were washed with MEM and subjected to a 25% glycerol/MEM shock for 1 min; 48 h later, cells were washed in Tris-buffered saline, and scraped from each plate. An aliquot was used for Hirt extracts²³. Hirt supernatants were dot-blotted onto nitrocellulose⁴⁰ and hybridized with a nick-translated pAT1.2 insert (specific activity 3×10^8 d.p.m. µg⁻¹). Hybridizations were carried out overnight at 68 °C in a solution consisting of 6×SSC, 5× Denhardt's solution, 1% SDS, 10 mM phosphate buffer pH 7.0, 1 mM EDTA, 100 µg ml⁻¹ sonicated salmon sperm DNA, 10% dextran sulphate (Pharmacia) and 10^6 d.p.m. ml⁻¹ of nick-translated probe. The remaining cells were subjected to one cycle of freeze-thawing and then sonicated in 100 µl 250 mM Tris-HCl, pH 7.8. In the case of COS-1 cells, 20 µl aliquots of clarified extract were assayed for CAT activity, whereas in all other cases 50 µl was used. The assay system was that described by Gorman *et al.*²⁰, which relies on the conversion of ¹⁴C-chloramphenicol to its acetylated derivatives and their resolution by silica gel TLC.

modest levels above background when introduced into Alexander cells. These experiments suggested that two unrelated genes can be expressed in the same tissues if they share homology in enhancer regions.

It was possible that the effects seen with the AT-III or immunoglobulin gene fragments were the result of the introduction of cryptic promoter elements into the pA₁₀CAT₂ vector. To address this issue, I performed a series of primer extension experiments. An end-labelled CAT-specific *Eco*RI/*Pvu*II restriction fragment was strand-separated and hybridized with total RNA from COS-1 cells that had been transfected with CAT plasmids 48 h previously. The DNA/RNA hybrid was then extended with reverse transcriptase and the size of the product analysed by polyacrylamide gel electrophoresis (Fig. 4). Transcripts initiating at the SV40 early promoter should have extended products 307 bp in length, whereas those originating from upstream sequences should be longer. Transcripts initiated correctly in each of the plasmids tested regardless of whether they contained SV40, AT-III or immunoglobulin upstream sequences.

An understanding of the developmental and/or tissue-specific control of eukaryotic gene expression will ultimately require an appreciation of the various factors whose interactions influence transcription. Specific DNA sequences are known to affect gene transcription, presumably by serving in a manner analogous to classical prokaryotic promoters in allowing for binding of RNA polymerases^{25–29}. The more recent descriptions of enhancers have defined a new class of transcriptional control signals, often with tissue-specific regulatory capacity. That enhancers function irrespective of position and orientation^{1,27–29} has given rise to the notion that, by disrupting chromatin structure, they serve as preferred entry points for RNA polymerases which then scan adjacent DNA sequences until encountering a promoter ele-

ment^{30–32}. Here, I provide evidence for an enhancer element at the 5' end of the human AT-III gene. Its presence was initially inferred on the basis of homologies between the AT-III gene and the *J_κ-C_κ* introns of murine and human immunoglobulin genes which contain tissue-specific enhancers. In addition to their homologies to viral sequences, however, the AT-III and immunoglobulin *J_κ-C_κ* genes share more extensive homologies with one another. That these homologies occur in close physical association with the polyoma virus-like and viral core sequences suggests that they are involved in the tissue-specific regulation of AT-III expression.

Immunoglobulin genes function poorly, if at all, in nonlymphoid cells such as fibroblasts^{3,4}. However, because of the close structural homology between AT-III and *J_κ-C_κ* enhancers, I tested for functional homology by introducing a murine *J_κ-C_κ* enhancer into Alexander hepatoma cells and the AT-III enhancer into myeloma cells. In both cases increased CAT activity was seen although the magnitude of the enhancement was low. This may reflect a suboptimal interaction between the enhancers and tissue-specific factors³³ or the need for additional factors that are absent from the heterologous cells. The *J_κ-C_κ* enhancer showed low activity even in myeloma cells and is consistent with the findings of Picard and Schaffner⁵ who demonstrated that the murine *κ*-chain gene enhancer is only 5% as effective as heavy-chain gene enhancers in promoting heterologous gene transcription.

Extensive homologies among enhancers have not previously been reported. Laimins *et al.*¹⁰ and Weiher *et al.*¹¹ have noted a consensus core sequence (GTGG^{AAA}TTT^G) in several viral enhancer elements but additional sequence homology was not observed. Walker *et al.*⁹ reported that the sequence GTGGAAA was found in fragments with enhancer activity from the 5'-flanking region of human and rat insulin genes although the

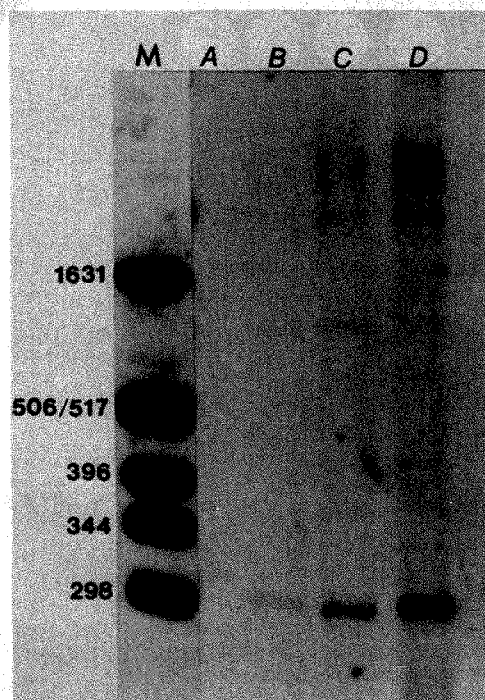


Fig. 4 A primer extension analysis of CAT transcripts in COS-1 cells. M, Marker. Primer extended products from cells transfected with: A, pA₁₀CAT₂; B, pA₁₀CAT_{2-K}; C, pATCAT-E3; D, pSV2CAT. **Methods.** Transfections were performed with 20 µg per plate of each plasmid tested (pA₁₀CAT₂, pSV2CAT, pATCAT-E3 and pA₁₀CAT_{2-K}). Cells were collected after 48 h and total RNA was extracted by the guanidine hydrochloride method³⁹. 25 µg RNA was hybridized with 80,000 d.p.m. of a 104-nucleotide-long EcoRI/PvuII strand-separated restriction fragment from the CAT gene which had been labelled at the EcoRI site to specific activity of 2 × 10⁷ d.p.m. µg⁻¹ using polynucleotide kinase and [γ-³²P]ATP (specific activity 7,500 Ci mmol⁻¹; Amersham). Hybridization conditions were: 80% formamide, 280 mM NaCl, 60 mM PIPES, pH 6.8 in 10-µl reactions at 51 °C for 16 h. Hybrids were then phenol-extracted, precipitated and redissolved in 25 µl 70 mM KCl, 10 mM Tris, pH 8.2, 8 mM MgCl₂, 10 mM dithiothreitol, 0.25 mM of each deoxynucleoside triphosphate and 50 U avian myeloblastosis virus reverse transcriptase. After 60 min at 42 °C, the reactions were phenol-extracted, precipitated, redissolved in formamide dye buffer and electrophoresed through an 8% polyacrylamide/8 M urea sequencing gel.

sequence was not required for optimal activity. Therefore, the homology between AT-III and J_κ-C_κ enhancers is striking in view of the minimal relatedness of other enhancers.

In an effort to correlate more precisely the J_κ-C_κ homology with enhancer activity, I have tested a series of deletion plasmids, one of which contains only 177 bp of AT-III sequence, including the immunoglobulin enhancer homology (nucleotide 153-330; Fig. 1). When tested in COS-1 cells, this construct is as active as any of the constructs shown in Fig. 2 (unpublished). Current experiments are focused on the construction of additional deletions to determine whether the same or different sequences are required for full expression in COS and Alexander cells.

The significance of the homologies reported here is not yet clear. It may be that cellular genes contain only a limited number of enhancer elements many of which have fairly extensive homology with each other despite widely different functions of the genes they control. AT-III and light-chain genes might fortuitously represent members of one such subset of enhancers. Alternatively, as B cells are thought to originate in the liver during early embryogenesis^{34,35}, these enhancers might represent common tissue- or stage-specific targets for cellular transcription factors. A distinction between these and other alternatives should be possible when more cellular enhancers have been characterized. In either case, it seems unlikely that the

homologies noted here represent the entire region responsible for tissue specificity. Despite the fact that the AT-III and J_κ-C_κ enhancer elements can function in heterologous cells, it is clear at least for immunoglobulin genes that this is not normally the case³⁶. Other unique cellular factors and/or DNA sequences are likely to be responsible for conferring additional tissue specificity on gene expression.

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Local cytoplasmic calcium gradients in living mitotic cells

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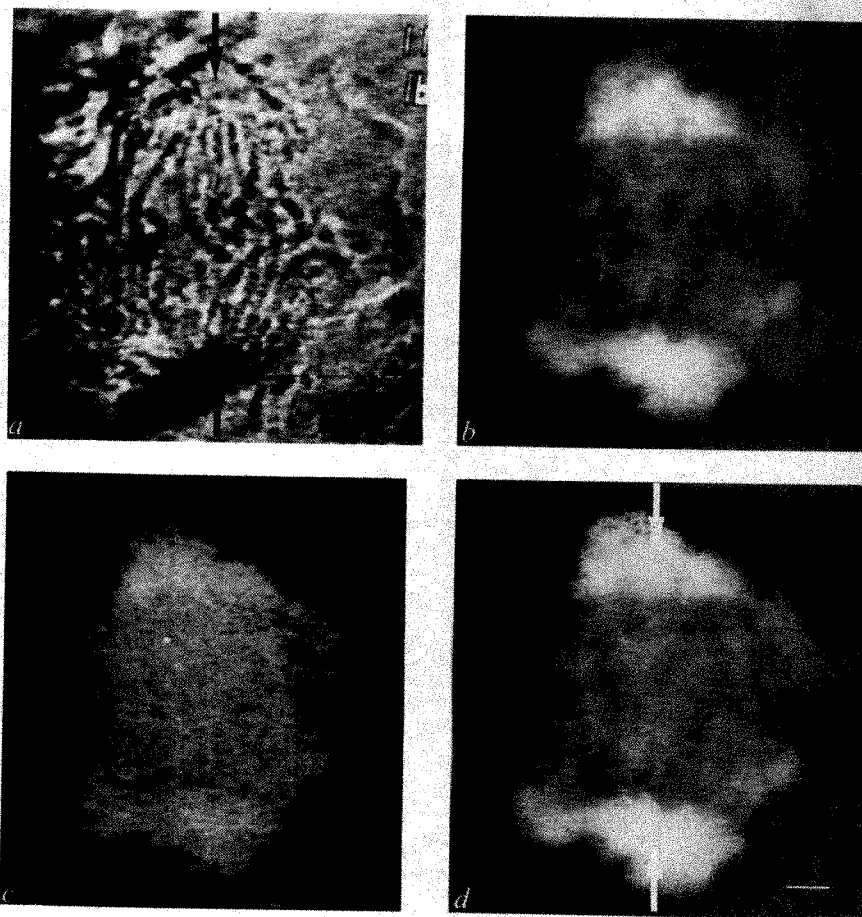
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Cytoplasmic free calcium has been proposed as a regulator of many microtubule-mediated processes, including mitosis. It has been difficult to test this hypothesis because methods for local measurement of free Ca²⁺ in the living cell have not been available. We have used the fluorescent calcium indicator dye Quin-2 (methoxyquinoline-1bis(o-aminophenoxy)ethane-N,N',N',N'-tetraacetic acid), which allows such observations to be made by digital processing of fluorescent images from the light microscope. Here

Fig. 1 Localization of calcium at the mitotic spindle poles of endosperm cells during mitosis. *a*, Bright-field image of a cell in anaphase; *b*, *c*, the same cell observed with 340 and 360 nm excitation after loading with Quin-2; *d*, the I_{340}/I_{360} ratio calculated at each point and redisplayed as an image of the ratio values. Bright areas in *d* correspond to areas of high $[Ca^{2+}]$. Cell wall-free endosperm cells of *Haemanthus Katherinae Baker* were expressed from young ovules onto glass coverslips coated with a thin film of Phytoagar (Gibco), overlain with a thin film of Gelrite (Kelco)²⁷ and perfused with 10 mM citrate buffer, pH 5.2, containing 1 mM $MgCl_2$, 3% glucose and 30 μM Quin-2/AM, for 20 min. Parallel experiments were carried out in medium supplemented with 0.1 mM $CaCl_2$. After loading, the cells were rinsed and incubated in perfusion buffer without the dye for 15 min and cells observed on a Diavert microscope (Leitz) modified for near-ultraviolet excitation¹⁴. Fluorescence-excitation light was passed through narrow band-pass filters centred at 340 and 360 nm, and emitted light was passed through a 500-nm broad bandpass filter (Oriel). (The image-analysis system has been described previously²⁸.) Fluorescent images were obtained with a Dage-MTI model 65 SIT image-intensifier video camera and were recorded on a Panasonic NV8030 videotape recorder with the automatic gain control suppressed. The camera was modified to linearize the intensity response. Images were digitized using a CAT-800 image digitizer (Digital Graphics) housed in a Horizon microcomputer (North Star Computers). The digitizer divides the image into 484×512 picture elements pixels and assigns intensity levels from 0 to 255 using a high speed A to D converter. Six video frames were averaged for each image to reduce noise, and the averaged images were transferred to an LSI 11/23 microcomputer (Digital) with a Gould IP8400 image analyser for further processing. Background values were determined by digitizing images of regions which contained no cells and subtracting these images pointwise from the images of cells. Cell-associated autofluorescence was <10% of the Quin-2 fluorescence with 340 nm excitation. The background-corrected images were then divided pointwise to provide an I_{340}/I_{360} image. The regions corresponding to the cell were identified using a threshold intensity in the 360 nm image, and the I_{340}/I_{360} image of the cell is shown in *d*. The areas of high cytoplasmic Ca^{2+} (high values of I_{340}/I_{360}) correspond to the mitotic spindle poles (arrows). The average cytoplasmic $[Ca^{2+}]$ is ~2-3-fold higher at the spindle poles than in the interchromosomal region. Similar values were observed in 19 other anaphase cells. Scale bar, 100 μm .



we report the application of this technique to the study of local Ca^{2+} concentrations in mitotic endosperm cells of *Haemanthus sp.*, and show that there is transient increase in free Ca^{2+} at the mitotic spindle poles during anaphase. This locally high Ca^{2+} may provide a mechanism for the regional control of microtubules and other cytoskeletal elements during anaphase.

Despite intensive study, the mechanisms of cell division remain poorly understood¹⁻³. Actin filaments⁴ or microtubules⁵ have been suggested as mediators of chromosome movement during mitosis, but the precise role of these cytoskeletal elements is unclear. Several lines of evidence suggest that cytoplasmic Ca^{2+} is important in the regulation of mitosis. Conditions that lower cytoplasmic Ca^{2+} delay the onset of anaphase⁶, and microinjection of Ca^{2+} during metaphase stimulates the transition to anaphase⁷. During anaphase, the spindle pole-to-chromosome microtubules become shorter; in echinoderms these spindle microtubules are very sensitive to Ca^{2+} during anaphase⁸. Calmodulin increases the sensitivity of microtubules to Ca^{2+} both *in vitro*⁹ and *in vivo*¹⁰, and high concentrations of calmodulin have been observed in the mitotic spindle of animal^{11,12} and plant¹³ cells during anaphase. These data suggest a regulatory role for Ca^{2+} in the shortening of the half-spindle, possibly acting through its effect on microtubules.

To determine whether cytoplasmic Ca^{2+} acts as a regulator of mitosis, accurate measurements of changes in Ca^{2+} must be made at each stage of mitosis. Because of the difficulty in obtaining tightly synchronized populations of dividing cells, such measurements must be made on individual cells assigned to a stage of mitosis by direct observation at the time of measure-

ment. Recently, a method has been developed for the measurement of cytoplasmic Ca^{2+} in individual cells using the fluorescent dye Quin-2 (ref. 14). The method is based on the observation that Quin-2 fluorescence excited at 340 nm is highly Ca^{2+} dependent, whereas the Quin-2 fluorescence excited at 360 nm is independent of $[Ca^{2+}]$ (ref. 15). Thus, the ratio of the two intensities (I_{340}/I_{360}) is dependent on Ca^{2+} but independent of changes in dye distribution resulting from changes in cell shape and thickness during mitosis. We have used this method to measure the average cytoplasmic $[Ca^{2+}]$ in rat kangaroo epithelial cells. During mitosis, the average cytoplasmic $[Ca^{2+}]$ was half the interphase value (28 against 53 nm), with no statistically significant variations observed among the mitotic stages¹⁶. These measurements of average $[Ca^{2+}]$ indicated that cytoplasmic Ca^{2+} does change during mitosis, but no evidence was obtained concerning local changes in $[Ca^{2+}]$ which might be required for directed motion. Here we report measurements of local $[Ca^{2+}]$ in mitotic cells.

The intensity ratio method is well suited for measuring local $[Ca^{2+}]$ by image digitization and analysis (see Fig. 1 legend). The intensity values provide a two-dimensional representation of cytoplasmic $[Ca^{2+}]$ within the cell. The horizontal resolution of the method is limited by the thickness of the cell and the optical properties of the microscope. Although the method has been developed using Quin-2, it is suitable for use with dyes of higher quantum efficiency and more favourable spectroscopic qualities, such as Fura-2 (ref. 17).

We used endosperm cells of the African blood lily, *Haemanthus Katherinae Baker*. The cells are extremely large, facilitating

observation of gradients, and their mitosis has been studied extensively¹⁸. The acetoxymethylester of Quin 2 (Quin-2/AM) was added to the cells (Fig. 1), which trapped Quin-2 by conversion of the ester to the tetracarboxylic acid. Cell-associated fluorescence increased ~10-fold with 340 nm excitation and 492 nm emission. During loading, the chromosome movement seems to stop. However, this inhibition is found to persist only for 5 min after removal of Quin-2/AM, after which anaphase progresses normally. For cytoplasmic Ca^{2+} measurements, the cells were incubated in buffer lacking Quin-2/AM for at least 15 min before the observation. Based on observation of several hundred cells, the loading procedure does not block the progression of cells from metaphase to telophase.

Criteria for selection of cells for measurements included: (1) flatness; (2) low refractive index; (3) no starch grains; (4) no debris or other cells covering the cell of interest; (5) observable chromosome movement in anaphase cells. Cells were observed, and I_{340}/I_{360} ratio images obtained, at all stages of mitosis. In interphase cells, the cytoplasmic $[\text{Ca}^{2+}]$ is fairly uniform. However, the cytoplasm of the ratio image is 1.6 times brighter than the nucleus. It is not clear that this can be attributed to a lower $[\text{Ca}^{2+}]$ in the nucleus as the behaviour of Quin-2 in the nuclear environment cannot be readily predicted. It is unlikely that the lower value results from exclusion of dye from the nucleus; when cells are labelled in prometaphase and allowed to proceed through telophase, the newly formed daughter nuclei also have a lower I_{340}/I_{360} fluorescence ratio. The prometaphase cell also exhibits a low ratio in the area of the nucleus despite the breakdown of the nuclear membrane. Cytoplasmic free Ca^{2+} is still evenly distributed. The metaphase cell does not show any readily observed inhomogeneity of $[\text{Ca}^{2+}]$ either along the spindle itself or outside the spindle area.

In anaphase, there is a marked departure from the homogeneous distribution of free Ca^{2+} seen in the previous stages. In 24 out of 28 anaphase cells examined, we observed regions of higher free Ca^{2+} near the mitotic spindle poles (Fig. 1). This distribution is observed in cells in early, mid and late anaphase, indicating that the gradient lasts from 30 to 45 min. Quantitative analysis in 20 of these cells indicated that the I_{340}/I_{360} ratio at the polar region was 1.67 ± 0.08 (s.e.m.) $\times$ higher than the ratio in the region between the chromosomes. This contrasts sharply with metaphase where the I_{340}/I_{360} ratio at the poles was only 1.1 ± 0.04 higher than at the metaphase plate. Eight anaphase cells were excluded from quantitative analysis for the following reasons: (1) Four cells, which did not demonstrate localization at the poles, were not flat. As the thickness of the cell becomes greater, contributions from out-of-focus parts of the cell may interfere with local measurements of intracellular Ca^{2+} ; (2) Four other cells which demonstrated localization of Ca^{2+} at the spindle poles were excluded because the Quin-2 fluorescence was either too high or too low for accurate measurements. In telophase, the cytoplasmic free Ca^{2+} distribution is once again more homogeneous, with lower I_{340}/I_{360} in the daughter nuclei.

To investigate the possibility that in cells loaded with Quin-2, citrate, a Ca^{2+} chelator, caused changes in cytoplasmic distribution, we compared cells incubated in low Ca^{2+} and Ca^{2+} supplemented medium (0.1 mM). In all stages of mitosis the pattern for that stage when incubated in high Ca^{2+} was not significantly different from that of the cells incubated in low Ca^{2+} medium. Sixteen anaphase cells were observed in low Ca^{2+} medium and the intensity ratio at the poles to that of the mid-zone was 1.66 ± 0.07 . Four additional anaphase cells were observed in high Ca^{2+} , and the ratio of Quin-2 fluorescence at the poles to that of the mid-zone (1.68 ± 0.27) was not significantly different from that of the low Ca^{2+} medium. In parallel experiments, whole-cell values of cytosolic Ca^{2+} in cells incubated in high Ca^{2+} medium (52 ± 7 nM, $n=4$) were similar to cells incubated in low Ca^{2+} medium (47 ± 4 nM, $n=11$). These observations

support the existence of large stores of intracellular Ca^{2+} and the existence of efficient Ca^{2+} homeostatic mechanisms in *Haemaphysalis endosperm*.

Our data indicate that cytoplasmic Ca^{2+} is elevated in the cytoplasm near the spindle poles during anaphase. Membrane-limited organelles have been observed along the mitotic spindle^{19,20} which may sequester Ca^{2+} , presumably to release it during anaphase^{21,22}. Our data show that the rate of release into the cytoplasm (and removal elsewhere) is sufficient to maintain a twofold gradient of $[\text{Ca}^{2+}]$ across the cell. Higher concentrations are probably attained near the sites of release, but our measuring procedure averages I_{340}/I_{360} throughout the thickness of the cell. The effect of Quin-2 itself on the gradient is not known, but Quin-2 probably reduces the magnitude of the $[\text{Ca}^{2+}]$ gradient by acting as a Ca^{2+} chelator.

Although the effect of local Ca^{2+} gradients during anaphase is not yet known, certain hypotheses can be suggested. Binding of Ca^{2+} reduces the mobility of calmodulin in the cell¹⁰, probably by causing binding of Ca^{2+} -calmodulin to relatively immobile proteins. Thus, locally high Ca^{2+} may trap calmodulin in the mitotic spindle, leading to the observed inhomogeneous distribution of calmodulin during anaphase¹¹⁻¹³. Ca^{2+} -calmodulin causes disassembly of microtubules^{9,10}, and the high concentrations of Ca^{2+} and calmodulin would be expected to cause the shortening of the kinetochore fibres as the chromosomes approach the poles. Of course, Ca^{2+} is also a regulator of actin-based force-generating systems²³, and locally high Ca^{2+} may activate such a system to provide a motile force.

Transient intracellular Ca^{2+} gradients have been reported for several phenomena, ranging from nerve-cell depolarization²⁴ to sea urchin egg activation²⁵. We have shown that long-lasting Ca^{2+} gradients exist in dividing cells during anaphase which may provide a mechanism for local control of motility within the cells. For example, microtubules could be disassembled by Ca^{2+} calmodulin in one region (for example, the mitotic spindle) while microtubule assembly proceeds elsewhere (for example, the interpolar microtubules)²⁶. These studies are now being extended to other cell types, particularly mammalian cells.

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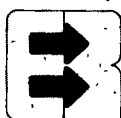
Montreal: (514) 849-5357. 1115 Sherbrooke St. W. H3A 1H3

Calgary: (403) 265-8780. 700-4th Ave. S.W. T2P 3J4

Vancouver: (604) 669-3550. 999 W. Hastings St. V6C 2W2

Ottawa: (613) 238-3256. 280 Albert St. K1P 5G8

(NW198)A



The Caldwell Partners International

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Define the leading edge in biotechnology

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This key executive will quarterback R&D on carbohydrate synthesis, therapeutic pheresis, molecular biology in the area of blood, bacterial and cancer diagnostics and anti-inflammatories. The V.P., R&D will also advise senior management on long-term research goals and establish close ties with academic research allies.

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Vancouver: (604) 669-3550. 999 W. Hastings St. V6C 2W2

Ottawa: (613) 238-3256. 280 Albert St. K1P 5G8

(NW199)A



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UNIVERSITÄT STUTTART

Zentrum Bioverfahrenstechnik

Am Standort Stuttgart wird im Verbund zwischen der Universität Stuttgart und der Fraunhofer-Gesellschaft ein Schwerpunkt Bioverfahrenstechnik aufgebaut.

Im Rahmen dieses Schwerpunktes wird an der Universität Stuttgart ein gut ausgestatteter Kernbereich, bestehend aus den Disziplinen Mikrobiologie, Genetik, Biochemie und Bioverfahrenstechnik eingerichtet, in dem in enger Kooperation Projekte gemeinsam ausgewählt und betrieben werden sollen.

Zu besetzen sind folgende Stellen:

In der Fakultät Chemie eine

C-4 Professur für Technische Biochemie

Der Bewerber soll in der Lage sein, das Fach Biochemie in der Lehre abzudecken. Der Schwerpunkt der Lehre ist die technische Anwendung der Biochemie. Der Bewerber soll auch Ausbildungsfunktionen für Biologen und Verfahrenstechniker übernehmen.

In der Fakultät Verfahrenstechnik eine

C-4 Professur für Bioverfahrenstechnik

Der Bewerber soll hervorragend ausgewiesen sein und über eigene Erfahrungen auf den Gebieten der Reaktionskinetik, der mathematischen Modellierung, der experimentellen Untersuchung und Analyse bioverfahrenstechnischer Prozesse verfügen. Vorausgesetzt werden ferner Aufgeschlossenheit und Erfahrung bezüglich ingenieurtechnischer Problemstellungen und — Lösungen in der Bioverfahrenstechnik. Industriearbeit wird begrüßt.

Bewerbungen mit Lebenslauf, Schriftenverzeichnis und Angaben über die bisherige Tätigkeit werden bis zum 15. Oktober 1985 erbeten. Sie sind für die erstgenannte Stelle zu richten an den Dekan der Fakultät Chemie, Pfaffenwaldring 55, D — 7000 Stuttgart 80, für die letztgenannte Stelle an den Dekan der Fakultät Verfahrenstechnik, Pfaffenwaldring 9, D — 7000 Stuttgart. (W2160)A

POSTDOCTORAL POSITION IN SOLID STATE PHYSICS WITH POSITIVE MUONS ETH ZÜRICH/SWITZERLAND

An open postdoctoral position has become available in the group for Muon Spin Rotation (μ SR) Spectroscopy at the *Institute for Intermediate Energy Physics of the ETH Zurich (IMP)*. The research program of this group involves the study of magnetic and electronic properties of metals, metal alloys and metal hydrides, ranging from simple metals to heavy Fermion systems.

An experimental physicist (Ph.D.) is sought, who is particularly inclined towards experimental work and who is willing to work in a team of 4-5 physicists and other outside collaborators. A strong solid state background or experience in NMR and related methods would be of advantage. The interdisciplinary character of the μ SR-research requires an aptitude in the use and handling of quite different equipment in a variety of measuring tasks (modern fast counting electronics, on-line computing, precision measurements of magnetic fields (NMR), cryostats (including a ^3He - ^4He dilution refrigerator), ovens, temperature measurements, physical characterization of samples e.g. by X-ray and neutron diffraction).

The appointment will be initially for three years with the possibility of extension for another three years. Additional information can be obtained from Dr A. Schenk (Telephone 056/99 32 63).

Applications, containing curriculum vitae, list of publications and references should be sent to:

SIN, Personnel Division
CH-5234 Villigen/Switzerland
Code IMP/31E

(W2155)A

Directorate of Planning Services (Minerals Division) Department of the Environment - London

Geologist

The Minerals Division of the Directorate of Planning Services is responsible for the Department's policy and professional advice on minerals and this post will deal with all non-energy minerals.

A major part of the work relates to aggregates. You will represent the Department on a number of Aggregate Working Parties, which are made up of county planning officers and representatives of industry, and act as technical secretary at a national level. You will be concerned with geological and minerals planning research carried out in support of the Department's policy functions and you will provide professional and technical advice for Ministers, senior civil servants and colleagues.

You must have a good honours degree in an appropriate scientific subject and at least 4 years' relevant postgraduate experience.

Appointment as Senior Scientific Officer £11,135 - £14,015 (including £1365 Inner London Weighting) with starting salary according to qualifications and experience.

RELOCATION EXPENSES MAY BE AVAILABLE

For further details and an application form (to be returned by 20 September 1985) write to: Department of the Environment, Room 459, Lambeth Bridge House, London SE1 7SB or telephone 01-211 6466. Please quote ref: SA/39/H.

The Civil Service is an equal opportunity employer

(7519)A

**Scientific
Civil Service**



CORPORATE BIOSCIENCE GROUP PLANT MOLECULAR BIOLOGY GROUP

Due to expansion of existing programmes the Plant Molecular Biology Group at ICI's Runcorn Laboratories have a staff vacancy for a

SCIENTIST with a strong background in PLANT TISSUE CULTURE

To join a team analysing plant gene expression using recombinant gene technology and plant transformation. Applicants should have a good Honours degree in one of the biological sciences, and at least two years of practical experience in plant tissue culture. Knowledge of plant transformation using Agrobacterium vectors would be a definite advantage.

The job is available immediately. Initially, the position will be held at Runcorn but within the next three years the group will transfer to a new Exploratory Plant Sciences Laboratory at ICI Plant Protection Division in Berkshire. Further information can be obtained from Dr. W. Schuch at Runcorn (0928) 511253.

The initial salary will depend on qualifications and experience, but will be in the range of £8,300-£9,300. Applications including names of two referees quoting ref. PMBG to:

Mr. R. Neale,
ICI Corporate Bioscience Group,
IMPERIAL CHEMICAL INDUSTRIES PLC.,
PO BOX 11, The Heath, Runcorn, Cheshire.

Closing date 19th September 1985

UNIVERSITY OF KENT**AT CANTERBURY ■■■■
BIOLOGICAL LABORATORY
POSTDOCTORAL
RESEARCH FELLOW**

Applications are invited for a Post-doctoral Research Fellow to join the new University of Kent — Laboratory of the Government Chemist BIOTRANS project. The post, initially for one year with the possibility of an extension for a further two years, is to investigate the biotransformation of halogenated xenobiotic compounds in the laboratories of Professor A T Bull and Dr D J Hardman. Experience in microbial physiology, biochemistry or biocatalyst immobilization would be an advantage. The salary will be £7520 – £12150 (under review) on the Grade 1A scale. Apply by letter with full curriculum vitae citing the names and addresses of two referees to the Assistant Registrar, Faculty of Natural Sciences, The Registry, University of Kent, CANTERBURY, Kent CT2 7NZ by 20 September 1985. Please quote ref A52/85/N (7511)A

**RESEARCH
ASSOCIATE
POSITIONS**

Positions are now available to study the mechanisms by which peptides obtained from eggs stimulate and/or attract spermatozoa using the sea urchin as a model system.

Positions are also available to pursue similar studies in higher animals including the mammal. Stipends will be commensurate with experience.

Please send curriculum vitae and references to: Dr David L. Garbers, Howard Hughes Medical Institute at Vanderbilt University Medical Center, 711 Light Hall, Nashville, TN 37232. (NW182)A

Biotechnologists

Beecham Pharmaceuticals is a highly successful company with an international reputation for excellence in research and development. Fermentation and enzymatic processes are used in the manufacture of several of the company's products. Improving the efficiency of these processes in order to reduce manufacturing costs is vital for success in today's highly competitive environment and requires an innovative approach, including the application of genetic engineering. These

functions are carried out by multi-disciplinary teams in the Fermentation Development Department which contains well-equipped laboratories and pilot plants.

The following job opportunities have arisen for which the successful candidates must demonstrate a strong motivation in applying their scientific discipline towards industrially-orientated objectives.

Fermentation Pilot Plant Support Laboratory

A Biochemist is required for the Fermentation Pilot Plant Technical Support Section to organise the work of a group of technical assistants. The work will involve participation in planning and monitoring experimental fermentations including data collection and processing as well as directing laboratory

investigations associated with these fermentations.

Applicants should possess a good honours degree in Biochemistry, preferably with additional experience in the study of fermentation processes.

Minifermenter Unit

A vacancy has also arisen in our Minifermenter Unit for a Development Scientist. Working in a well-equipped department, the successful candidate will contribute to the development of existing and new fermentation processes and will be closely involved in the use of modern

instrumentation and computers for data logging and process control. Candidates should have a good honours degree; previous experience of fermentation and computers would be useful, but not essential.

The Beecham Group offer attractive starting salaries backed by excellent conditions of service, including a non-contributory pension and life assurance scheme. Worthing is situated in a pleasant coastal area with many amenities and easy access to the surrounding countryside. Assistance with relocation expenses will be given if appropriate.

Please write or telephone, indicating which job you wish to apply for to: Mr R P Reynolds, Senior Personnel Officer, Employee Relations Department, Beecham Pharmaceuticals, Clarendon Road, Worthing, West Sussex BN14 8QH. Tel: Worthing (0903) 39900, ext 332.

Beecham Pharmaceuticals

(7491)A

**CELL BIOLOGY
AND PHYSIOLOGY
WASHINGTON UNIVERSITY SCHOOL
OF MEDICINE
ST LOUIS, MISSOURI**

The Department of Cell Biology and Physiology, Washington University School of Medicine, St Louis, Missouri, is expanding its commitment to research in the following areas: (i) membrane protein function and biogenesis; (ii) assembly, function, and movement of intracellular organelles; (iii) cell motility and contractility; and (iv) cell-cell and cell-matrix interactions. Individuals who employ novel biophysical and recombinant DNA methodologies are especially encouraged to apply. Tenure track positions are available for successful candidates who will be expected to develop an independent research program and to participate in the training of medical and graduate students.

Please submit curriculum vitae and the names of three references to: Dr Philip Stahl, Department of Cell Biology and Physiology, Washington University School of Medicine, 660 South Euclid Avenue, St Louis, MO 63110.

An Affirmative Action / Equal Opportunity Employer.

**BIOCHEMIST/
MOLECULAR BIOLOGIST**

The Department of Biochemistry and Biophysics is seeking applications for two Assistant or Associate Professorships. These are 12 month, tenure track positions jointly funded by the College of Agriculture and the Texas Agricultural Experiment Station. Outstanding individuals having research interests dealing with the molecular aspects of regulation, structure, mechanism or function in microbial, animal or plant systems are sought. In addition to research, participation in the department graduate and undergraduate teaching programs will be required. A terminal degree in either biochemistry or a related field plus appropriate postdoctoral experience is required.

Applicants should send a curriculum vitae, list of publications, a brief description of current and future research directions, and names of three references to:

Dr. E. G. Sander, Head
Department of Biochemistry and Biophysics
Texas A&M University
College Station, TX 77840-2128

Applications will be accepted until October 15, 1985, or until suitable candidates are found.

The Texas A&M University System is an Affirmative Action/Equal Opportunity



UNIVERSITÄT STUTT GART

Zentrum Bioverfahrenstechnik

Am Standort Stuttgart wird im Verbund zwischen der Universität Stuttgart und der Fraunhofer-Gesellschaft ein Schwerpunkt Bioverfahrenstechnik aufgebaut.

Im Rahmen dieses Schwerpunktes wird an der Universität Stuttgart ein gut ausgestatteter Kernbereich bestehend aus den Disziplinen Mikrobiologie, Genetik, Biochemie und Bioverfahrenstechnik eingerichtet, in dem in enger Kooperation Projekte gemeinsam ausgewählt und betrieben werden sollen.

Als eine der zu besetzenden Stellen ist in der Fakultät Geodätische Biowissenschaften eine

C4 — Professur für Industrielle Genetik

zu besetzen.

In der Lehre ist die Grund- und Fortgeschrittenenausbildung im Fach Mikrobielle Genetik und Gentechnologie für Studenten der Biologie, Biochemie und Bioverfahrenstechnik zu erbringen.

Bewerbungen mit Lebenslauf, Schriftenverzeichnis und Angaben über die bisherige Tätigkeit sind bis zum 15. Oktober 1985 an den Dekan der Fakultät für Geo- und Biowissenschaften, Biologisches Institut, Ulmer Str. 227, D 7000 Stuttgart 60 zu richten. (W2161)A

NORDITA COPENHAGEN

ASSISTANT PROFESSOR IN THEORETICAL NUCLEAR PHYSICS

NORDITA, the Nordic theoretical physics institute, located at the Niels Bohr Institute of Copenhagen University, has an opening starting in January 1986, or later if that is more convenient, for an assistant professor in theoretical nuclear physics. Nuclear physics will be interpreted in the broadest terms including the areas of nuclear structure, intermediate energy physics, the effects of quarks and other degrees of freedom in nuclear phenomena, as well as the phenomena to be expected in ultra relativistic collisions of heavy nuclei.

The successful applicant is expected to guide fellows at roughly the postdoctoral level and to interact with colleagues at NORDITA and elsewhere in the Nordic countries. The position provides excellent opportunities to pursue original research and to have contact with a wide range of developments in theoretical physics. There are good facilities for travelling to other institutes and to meetings outside the Nordic countries; and the assistant professor will be encouraged to invite guest scientists to NORDITA.

The initial appointment will be for three years, with the possibility of renewal up to a total of six years. The annual salary will be in the range of 190,000–270,000 Danish Kroner depending on experience.

Those interested in the appointment should send curriculum vitae, a list of publications and the names of three referees before 1 November 1985 to the Director, Nordita, Blegdamsvej 17, DK-2100 Copenhagen Ø, Denmark. There is no restriction on the nationality of the applicant. Those wishing to recommend suitable candidates are urged to contact the Director. (W2151)A

METEOROLOGICAL ANALYST BUREAU OF METEOROLOGY RESEARCH CENTRE Melbourne Australia

SALARY \$A26,056 — \$A28,831

Applications for the position of Meteorological Analyst in the recently created Bureau of Meteorology Research Centre (BMRC) are invited from Scientists with research experience in the field of atmospheric modelling to conduct research in the Medium Range Prediction Group. The successful applicant will be expected to:

- (i) develop procedures to diagnose and evaluate numerical model performance; and
- (ii) identify the physical and dynamical mechanisms associated with errors in numerical forecasting models.

Qualifications

An appropriate degree or diploma in Science or equivalent qualifications. Research experience in the fields of atmospheric modelling and computational physics is desired. Possession of a PhD is highly desirable.

Conditions

The successful applicant will be offered an initial fixed term of engagement for a period of three years. Re-engagement for a further period may be available. Conditions of service include four weeks annual recreation leave, cumulative sick leave, three months long service leave after ten years, an annual leave loading and a comprehensive superannuation scheme. Assistance with removal costs will also be available, if required.

Enquiries may be directed to Dr W. Bourke, Senior Principle Research Scientist (BMRC). Telephone: Melbourne (03) 669 4425. Applications stating full personal and professional details and the names of referees should be forwarded to:

**DIRECTOR OF METEOROLOGY
GPO BOX 1289K
MELBOURNE, VICTORIA 3001
AUSTRALIA**

(W2156)A

UNIVERSITY OF MARYLAND SCHOOL OF MEDICINE

Department of Biological Chemistry

POSTDOCTORAL RESEARCH ASSOCIATE

Position is available for three years from September 1, 1985 to study molecular mechanisms of hormonal regulation of heart Ca^{2+} channels. Research involves receptor binding, protein phosphorylation, Ca^{2+} flux, and electrophysiological studies using cultured heart cells. Background in biochemistry, cell biology, or pharmacology is essential with a commitment to training in biochemical approaches. Salary negotiable.

Applications along with CV and the names of three references should be sent to: Dr. Terry B. Rogers, Dept. of Biochemistry, Univ. of Maryland, School of Medicine, 660 W. Redwood St., Baltimore, MD, 21201.

Equal Opportunity Employer.

(NW187)A

Director, Hopkins Marine Station Department of Biological Sciences STANFORD UNIVERSITY

The Department of Biological Sciences of Stanford University seeks a distinguished biologist to serve as professor and director of the Hopkins Marine Station. The director will hold an endowed chair and will be appointed as Professor of Biological Sciences at Stanford University. The director will be expected to provide scientific leadership with an established, vigorous research program in the area of cell, developmental, molecular, environmental, organismal, and/or population biology. The director's responsibilities include the provision of leadership for faculty expansion and for development of innovative teaching programs at the Station.

Applicants should submit a curriculum vitae and the names of three persons willing to serve as reference to:

**Chairman, HMS Search Committee
Department of Biological Sciences
Stanford University
Stanford, CA 94305**

Stanford is an equal opportunity employer.

(NW133)A

SUNDERLAND AND TEESIDE POLYTECHNICS

NORTH EAST BIOTECHNOLOGY CENTRE POSTGRADUATE RESEARCH ASSISTANT IN MOLECULAR GENETICS

Salary: £5,181-£5,910 (under review)

The North East Biotechnology Centre (NEBC) is a National Centre for Biotechnology based at Sunderland and Teesside Polytechnics. The Centre has a vacancy in the Biology Dept. at Sunderland Polytechnic for a Research Assistant to undertake a research project involving a molecular analysis of transposable element systems in maize. Post suitable for graduates in biochemistry, molecular biology or botany and experience with recombinant DNA techniques would be an advantage. Candidate will be expected to register for a higher degree.

An application form and further particulars obtained from the Personnel Officer, Sunderland Polytechnic, Langham Tower, Ryhope Road, Sunderland, SR2 7EE, or telephone (0783) 76231 Ext 11.

Closing date: 12 September 1985

(7506)A

RESEARCH ASSOCIATE IN PROTEIN CRYSTALLOGRAPHY

Postdoctoral or more senior position available for protein crystallographer to work on the structure determination of fibrinogen (principal fibrous protein in blood clotting) and troponin/tropomyosin (protein switch in muscle contraction). The Structural Biology Laboratory at Brandeis has exceptional facilities for X-ray diffraction, electron microscopy and image processing, and provides access to a broad range of problems and techniques in macromolecular structure determination.

Candidates with backgrounds in small molecule crystallography or image processing of electron micrographs will also be considered. Please forward résumé and names of three references.

Contact: Professor Carolyn Cohen, Rosenstiel Basic Medical Sciences Research Center, Brandeis University, 415 South Street, Waltham, MA 02254.

An equal opportunity/affirmative action employer. (NW176)A

MARIE CURIE MEMORIAL FOUNDATION RESEARCH INSTITUTE POST DOCTORAL RESEARCH POSITIONS

to study the role of the cellular p53 oncogene in multi-stage carcinogenesis.

1. The role of p53 in SV40 and Adenovirus transformation. Reagents available include p53 deletion mutants that bind SV40T or E1b55k but are defective for cellular immortalization, point mutants that fail to bind the viral oncogene products and p53 antisense retroviral vectors.
2. Investigation of p53 mutants that exhibit enhanced activity in cellular immortalization assays.

These three year positions are funded on the University Lecturer scale.

Applications, C.V. and the names of two referees to Dr. J. Jenkins, MCMF Research Institute, The Chart, Oxted, Surrey, GU8 3PP (0883 333333) (7445)A

Cell Physiologist (PhD)

elf ^{bio}
recherches

Elf Bio Recherches, a subsidiary of Elf Aquitaine and Sanofi, specialises in biotechnology. It is looking for a Cell Physiologist for its laboratory of animal cell physiology and fermentation. This laboratory (2 scientists and 3 technicians) forms part of the recombinant animal cell technology unit. The research activities of this group include the investigation of the role of physiological factors involved in the expression of genes cloned into animal cells. Candidates should have a profound knowledge of animal cell physiology. Experience in medium or large-scale tissue culture will be an advantage. Knowledge of French would be appreciated. Elf Bio Recherches R & D facilities are located in the Toulouse area, in Southern France. Curriculum vitae and list of publications should be sent to Mr Fabre quoting Reference B/T 2018N. (W2149)A

PA

71 bis, allées Jean-Jaurès, 31000 Toulouse, France.
Tel: Toulouse 63.70.63.

MANAGING DIRECTOR

Boyce Thompson Institute at Cornell University

Applications and nominations are invited for the position of Managing Director of Boyce Thompson Institute for Plant Research at Cornell University. Boyce Thompson Institute (BTI) is a non-profit, privately endowed organization conducting research on plants and plant pests. The Institute is located in a facility completed in 1978 on the campus of Cornell University and is affiliated with the College of Agriculture and Life Sciences. BTI has an annual budget of \$7-8 million and employs approximately 125 scientists and support staff, 60 at the Ph.D. level.

The Managing Director serves as chief executive officer and is responsible for formulation of Institute policy, evaluation of productivity of research programs, selection of scientific personnel, recruitment of research support and overall operation of the Institute and interaction with Cornell University administration.

Selection will be based upon experience consistent with the goals of the Institute, which include basic and applied research in the plant sciences relevant to national and world agriculture. Previous scholarly endeavor, organizational ability and success in recruiting research support will be considered.

Applicants should send curriculum vitae, bibliography and names of five professional people acquainted with the applicant's background to Dr. Leonard H. Weinstein, Chairman of the Screening Committee, Boyce Thompson Institute at Cornell University, Ithaca, NY 14853. Nominations should also be sent to Dr. Weinstein. All applications and nominations should be submitted as soon as possible but not later than October 15, 1985.

An Affirmative Action/Equal Opportunity Employer. (NW179)A

DIRECTOR (male or female)

vacancy number 5-2221/2407

The Royal Dutch Academy of Letters, Arts and Sciences for the "Centraalbureau voor Schimmelcultures" at Baarn, The Netherlands.

The "Centraalbureau voor Schimmelcultures" (CBS - Central Institute for Fungal Cultures), with its main Laboratory in Baarn and its smaller yeast department in Delft, is an institute for research into the taxonomy of fungi (including yeasts) and actinomycetes. The CBS also houses, and is responsible for the management of, an extensive collection of fungi, yeasts and actinomycetes. The institute employs 15 scientists and 26 other staff members.

First choice will be given to a taxonomic mycologist but microbiologists with relevant experience or interests will also be considered. The candidate will be expected to hold a doctorate. In addition to being an efficient manager the director should have outstanding scientific qualities. He/she coordinates the entire research programme of the Institute in addition to pursuing his/her own research. He/she also has final responsibility for financial and technical supervision. The position is located in Baarn.

Salary: depending on age and experience, max. Dfl 9495,- per month. Further details can be obtained from the secretary of the selection committee, Professor K. Verhoeff, Willie Commelin Scholten Phytopathological Laboratory, 20 Javalaan, Baarn, The Netherlands (tel. 02154-15654).

Please send your application for 13th September 1985 to the Rijks Psychologische Dienst, Postbus 20013, 2500 EA The Hague, Holland.

(W2162)A

SENSORY NEUROBIOLOGIST

The Department of Neurobiology and Physiology at Northwestern University is seeking applications for a faculty position, at the level of Professor or Associate Professor in the general area of Sensory Neurobiology. The successful candidate will have a strong, well-established program of research, preferably in developmental or cellular/molecular neurobiology dealing with one of the sensory systems.

The Department of Neurobiology and Physiology has strong, well-funded groups in Sensory Neurobiology and in Neuroendocrinology, both representing multidisciplinary approaches to these areas of Neuroscience. The Department currently administers a PhD program; two NIH training programs are centred in the department. The Department also has an undergraduate major leading to a BA, and a BA/MS honours program for exceptional undergraduates.

Interested persons should submit a curriculum vitae and the names of three persons who can provide references to:

Dr. Albert I. Farbman,
Department of Neurobiology and Physiology,
Northwestern University,
Hogan Hall, Evanston, IL 60201.

Northwestern University is an Equal Opportunity/Affirmative Action Employer. (NW167)A

BIOTECHNOLOGISTS!

What's going on in the world's fastest-growing scientific discipline?

To find out, send for a sample copy of **BIO/TECHNOLOGY**, the International Monthly for Biology and Industry, to

Felicity Parker, Macmillan Journals,
4 Little Essex St.,
London WC2R 3LF
or

Paul Siman, Bio/Technology,
65 Bleecker St., New York, NY 10012.

** SENIOR SCIENTIST **

The W. Alton Jones Cell Science Center, Inc., Lake Placid, New York has an immediate opportunity for an innovative and experienced molecular biologist to direct independent research in the field of molecular and cellular biology.

We are seeking a dedicated and talented scientific professional with interest in cell biology and growth factors. The successful candidate will have a doctorate in molecular and cellular biology with at least 3 years postdoctoral experience. Candidates must have the ability to supervise others. Liberal benefits. Salary will commensurate with experience.

Applicants are requested to submit complete curriculum vitae and to have three letters of academic references forwarded by October 1, 1985 to:

Personnel Manager
W. ALTON JONES CELL SCIENCE CENTER,
10 Old Barn Road
Lake Placid, New York 12946

AN AFFIRMATIVE ACTION/EQUAL OPPORTUNITY EMPLOYER

M.R.C. CLINICAL RESEARCH CENTRE
(NORTHWICK PARK HOSPITAL)
WATFORD ROAD, HARROW
MIDDLESEX HA1 3UJ



NON-CLINICAL SCIENTIST

Applications are invited from Electron Microscopists for this 3 year appointment in the Division of Clinical Cell Biology to investigate the uptake and intracellular processing of various ligands by hepatocytes with special reference to the involvement of lysosomes and related organelles. There will be collaboration with other scientific staff of the Division and the section of Histopathology in the use of EM facilities.

Salary will be within the range £10153-£16158 (inclusive of London weighting) depending on qualifications and experience.

For an opportunity to visit the Division contact Dr. T.J. Peters on 01-864 5311 Extn. 2139.

Application forms available from Betty Shaw, Personnel section quoting reference 104/1/3963. Closing date for completed application forms, 19th September, 1985. (7507)A

SENIOR RESPIRATORY PHARMACOLOGIST AT SEARLE

G.D. Searle is a major international Pharmaceutical Company whose Research & Development Division at High Wycombe has several exciting programmes in the field of chemically and biologically derived products.

We are seeking an experienced postdoctoral pharmacologist to lead a new team investigating the pathological mechanisms of human respiratory disease. An all round understanding of respiratory pharmacology is essential and he or she will be expected to contribute expert theoretical and practical knowledge to the project. Frequent interaction with other departments and external academic centres will be necessary. Relevant experience within an

industrial environment would be an advantage, but is not essential.

The Company offers competitive salaries, 23 days holiday, contributory pension fund, health insurance scheme, subsidised cafeteria, recreation club and modern offices in a very pleasant location. Generous relocation assistance will be available in appropriate cases.

Please apply to the Personnel Manager, Searle Research & Development, Lane End Road, Sands, High Wycombe, Bucks. (Tel. High Wycombe 21124 Ext. 3372). Please quote reference B1362.

(7522)A

SEARLE

UNIVERSITY OF ULM



DEPARTMENT OF CLINICAL PHYSIOLOGY AND OCCUPATIONAL MEDICINE

(Director: Prof. Dr. med. T.M. Fiedner)

There is a vacancy to be filled as soon as possible (of 4 to 5 years duration) in the Euratom-Project of radiation-hematology as a scientist (BAT II/b). Final signature of the contract is reserved. Candidates should have a Ph.D. or equivalent with a dissertation in a biomedical field as well as having experience with cell cultures, cell separation methods and/or analysis of regulating factors.

Please send your application along with a list of your publications, certificates, reference letters and curriculum vitae to — Prof. Dr. rer. nat. W. Nothdurft, Abteilung für Klinische Physiologie und Arbeitsmedizin der Universität Ulm, Oberer Eselsberg M 24, 309 D-7900 Ulm/Donau.

(W2164)A

LIVERPOOL SCHOOL OF TROPICAL MEDICINE

Wolfson Molecular Genetics Unit/ Venom Research Unit

RESEARCH ASSISTANT

to join a project, funded by the MRC, investigating the molecular biology of snake venom antigen genes. Applicants should have a good honours degree in a relevant subject; some experience of molecular biology would be an advantage.

The post will be for a period of 3 years with a starting salary of £6,600. The successful applicant would be encouraged to register for a Ph.D.

Applications, including full c.v. and the names of two referees, should be sent by 14 September to Dr J. M. Crampton, Wolfson Molecular Genetics Unit, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA.

(7512)A

SENIOR RESEARCH ASSOCIATE/ POST DOCTORAL ASSOCIATE

Applications are invited from interested persons for the positions of Senior Research Associate/Post Doctoral Associate in the Wits-CSIR Schonland Research Centre for Nuclear Science for initial appointments of 3 years.

The spectrum of research at the Centre is wide and embraces fundamental nuclear and atomic physics as well as the application of nuclear techniques in metallurgy, geology, mineralogy, hydrology and biology.

Further information can be obtained from Mr. Geoff Henning, South African Universities Office, Chichester House, 278 High Holborn, London WC1V 7HE. Interested parties should submit a detailed curriculum vitae, with names and addresses of 3 referees by 30th September 1985.

It is the policy of the University not to discriminate on the grounds of sex, race, colour or national origin.

(W2159)A



UNIVERSITY OF
WITWATERSRAND

MEDICAL RESEARCH COUNCIL

The MRC Tuberculosis and Related Infections Unit at the Hammersmith Hospital, London W12 0HS requires postdoctoral scientists for the following positions:

POSITION "A": A biochemist/molecular biologist interested in the structural analysis of antigenic determinants of mycobacterial proteins recognised by cloned T-cell subsets. Background experience in peptide and/or recombinant DNA technologies would be desirable.

POSITION "B": An immunologist interested in the immunomodulatory function of molecules isolated from mycobacteria cell walls. Background experience in immunobiology and immunogenetics in murine models would be desirable.

POSITION "C": An immunologist interested in genetic (HLA-D) requirements of cellular interactions in the human immune response to mycobacterial antigens. Background experience in lymphocyte tissue culture and/or HLA studies would be beneficial.

All three appointments are for a period of 3 years with a possibility of extension up to 5 years. Remuneration will be based on the University non-clinical academic staff scales. Applications should be submitted to the Director of the Unit, Dr. J. Ivanyi (address above, phone 01-743-4594, Ext. 67). (7493)A

MRC
Medical Research Council

Faculty Position available, tenure-track, in molecular genetics, with a special interest in membranes. Applicants should have research background in recombinant DNA technology (preferably also in somatic cell genetics) and working knowledge of membrane proteins. M.D. and/or Ph.D. in appropriate field required. Successful candidate will hold tenure-track appointment (Assistant or Associate Professor, depending upon qualifications) in Department of Physiology and Molecular Biophysics at Baylor College of Medicine. Joint appointments in relevant academic departments also possible. Laboratory space and basic equipment provided and initial laboratory support available. Successful candidate will devote majority of his/her effort to establishing and maintaining an independent research program related to the main interests of the Department. The Department is building a strong program in membrane biophysics with expertise in electrophysiology, membrane biochemistry, reconstitution, and modelling. **Send vita, a brief description of past and present research experience, and names of three or more references to**

**Dr. A. M. Brown, Incoming Chairman,
Physiology and Molecular Biophysics Department**



**Baylor College of Medicine,
One Baylor Plaza,
Houston, Texas 77030.**

An Equal Opportunity A/A Employer

(NW58)A

BIOCHEMIST

to study long-term biochemical regulation of membrane channels within an established multidisciplinary program investigating molecular substrates of learning and memory. High resolution, two-dimensional gel electrophoresis; HPLC, auto-radiography; enzymatic assays (eg. kinases, adenylate cyclase, phosphatase, etc); protein synthesis, recombinant DNA techniques; and artificial membranes are examples of desirable areas of expertise.

Candidates with a PhD and/or MD and at least two years post-doctoral experience preferred. Salary range \$25,000-\$35,000 with excellent benefits.

Submit resume to Personnel Office, Marine Biological Laboratory, Woods Hole, MA 02543 by September 20, 1985

The MBL is an Affirmative Action/Equal Opportunity Employer.

(NW181)A

CLINICAL SCIENTIST

Astra is an international pharmaceutical company with a strong commitment to research. The Astra Clinical Research Unit in Edinburgh has an opportunity for an experienced clinical scientist to participate in our expanding programme of clinical trials in the field of gastroenterology.

The successful candidate should be able to communicate effectively and possess a post-graduate qualification in a life science discipline, with ideally 2 or 3 years relevant experience in the pharmaceutical industry.

A competitive salary and benefits package will be offered.

Applications enclosing a detailed C.V. should be marked Confidential and addressed to:

**THE DIRECTOR OF CLINICAL RESEARCH
ASTRA CLINICAL RESEARCH UNIT
10 York Place
Edinburgh, EH1 3EP
SCOTLAND**

(7524)A

UNIVERSITY OF NOTTINGHAM

DEPARTMENT OF APPLIED
BIOCHEMISTRY AND
FOOD SCIENCE

Applications are invited for a
RESEARCH ASSISTANT

to work on neutral protease activity in muscle of farm species. The proposed starting date is November 1st 1985. The project is in association with a large international company and it is hoped to publish the results in the scientific literature. The project is planned to last three years and will be supervised by Dr R G Bardsley and Professor P J Buttery. Suitable applicants will have a thorough training in biochemistry or a related subject. Knowledge of protein separation techniques would be an advantage. Preference will be given to applicants with previous postgraduate experience. A desire to relate their studies to problems of agricultural importance would be a great advantage.

The Department has well equipped laboratories for biochemical studies with farm animals and it is situated on the Sutton Bonington Campus of the University, some 6 miles north of Loughborough.

Salary will be on the research and analogous staff scales with a starting salary up to £8,920 (under review) with USS benefits.

Applications giving the names of two academic referees and including a curriculum vitae should be addressed to the Secretary, University of Nottingham School of Agriculture, Sutton Bonington, Nr Loughborough, Leics, LE12 5RD to arrive as soon as possible. (7500)A

THE INSTITUTE OF CANCER RESEARCH, AND THE ROYAL MARSDEN HOSPITAL

CLINICAL RESEARCH
ASSISTANT/DATA
COLLECTOR

Applications are invited for the above post in the Section of Radiotherapy, Sutton, Surrey. The holder of the post will be particularly involved with handling clinical data pertaining to patients with malignant lymphoma, entering such data on to a computer terminal and working closely with clinicians and statisticians in the analysis of treatment results. The post would be suitable for a non-clinical science graduate.

Salary in the range of £5738 - £8096 p.a. + £648 p.a. Outer LWA depending on qualifications and experience.

Applicants are advised that in the majority of The Institute's premises, smoking is prohibited.

Applications in duplicate with the names and addresses of two referees should be sent to the Personnel Officer, The Institute of Cancer Research, 17A Onslow Gardens, London, SW7 3AL, quoting refer-

THE NATURE CONSERVANCY COUNCIL

Specialist in Scientific
Data-Handling

**HIGHER SCIENTIFIC
OFFICER/SENIOR
SCIENTIFIC OFFICER**

Applications are invited for the above permanent post within the Chief Scientist Directorate based at the Council's Great Britain House, Peterborough. The holder will be responsible for maintaining and developing computer systems for the general support of scientific staff and in the development of the computerisation of NCC's licensing records, as well as other duties.

Qualifications. Candidates should have a degree in an appropriate subject and at least 2-4 years post graduate experience. Essential expertise will include a sound knowledge of data management for scientific purposes and of scientific data analysis. Preference will be given to candidates with experience of managing biological information and analysis.

Salary, depending on qualifications and experience.

HSO £7,785 rising to £10,541

SSO £9,772 rising to £12,653.

For an application form and further details of the post please contact, Miss B A Meredith, Person I, Nature Conservancy Council, Northminster House, Peterborough PE1 1UA, to whom completed forms should be returned by September 1985.

The Nature Conservancy Council is an equal opportunity employer. (7504)A

CHARING CROSS MEDICAL RESEARCH CENTRE

IMMUNOLOGY UNIT
RESEARCH ASSISTANT

Applications are invited from workers with wide experience in biochemistry, immunochemistry and cellular immunology (in vitro and vivo assays) for a position as research assistant to work with Dr M Feldmann on a project to analyse mechanisms of HLA Class II antigen regulation in disease. Preference will be given to candidates with an interest in immune regulation and capable of independent research.

Appointment will be for 1 year first instance, with starting salary the appropriate point of Research 1A scale, depending on age, qualifications and experience.

Applications (with curriculum vitae, and name of two referees) should be sent to:

The Administrator, Charing Cross Medical Research Centre, Lurg Avenue, Hammersmith, London W6 8LW

Applications should arrive by.

THE AUSTRALIAN NATIONAL UNIVERSITY

Research School of
Earth Sciences

POSTDOCTORAL FELLOW/ RESEARCH FELLOW/ SENIOR RESEARCH FELLOW IN GEOPHYSICAL FLUID DYNAMICS

Applications are invited from suitably qualified men and women for appointment as Postdoctoral Fellow Grade 1, Research Fellow or Senior Research Fellow in Geophysical Fluid Dynamics in the Research School of Earth Sciences. Double-diffusive convection is a central theme of the research of the Geophysical Fluid Dynamics Group headed by Professor J S Turner. Candidates are sought with interests in one or both of the following fields: (a) Laboratory studies of fluid dynamical processes in the interior of the earth including layered magnetic intrusions, variable viscosity in magma mixing, the withdrawal of fluid from magma chambers, diapir motion and large-scale convection in the mantle; (b) Laboratory and theoretical studies of mixing in the ocean. Applications from candidates with backgrounds in petrology or tectonophysics who are interested in quantitative modelling of problems in category (a) would be particularly welcome.

Closing date: 27 September 1985.
Ref: ES 11/7/1.

Conditions of appointment: Salary will be in accordance with qualifications and experience within the range: Postdoctoral Fellow Grade 1 \$A22,614-\$25,905 pa; Research Fellow \$A26,236-\$34,467 pa; Senior Research Fellow \$A36,649-\$43,715 pa; current exchange rate: £A1 = UK51p.

Appointment will be: Senior Research Fellow/Research Fellow normally up to three years with the possibility of extension to maximum of five years; Postdoctoral Fellow normally two years, with the possibility of extension to maximum of three years.

Grants are provided towards travel and removal, though special conditions may apply to persons appointed to externally funded positions. Assistance towards housing is given to an appointee from outside Canberra. Eligible appointees will be required to join the Superannuation Scheme for Australian Universities. Maternity leave is available. The University reserves the right not to make an appointment or to make an appointment by invitation at any time.

Applications should be submitted to the Acting Registrar, Australian National University, GPO Box 4, Canberra, ACT2601, Australia. Applicants should quote the reference No. when submitting applications and when requesting further particulars which are also available from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

The University is an equal opportunity employer.

CAREER OPPORTUNITY ONCOGENE SCIENCE, INC.

Is seeking to expand its staff at Long Island, NY research facilities for programs in

**Oncogene and growth factor research
in order to develop products for early
detection -- monitoring -- treatment
of human cancer.**

— Position Available —

DIRECTOR OF DIAGNOSTIC R&D PROGRAMS

Responsibilities include management of basic research and product development programs for human cancer diagnostics including strategic R&D planning and coordination of contract research with corporate sponsors. Successful candidate will have Ph.D. with experience in management of research and development programs in industrial setting particularly in the area of immunodiagnostics; knowledge of oncogene/growth factor research would be an asset.

Send resume to Scientific Director, Oncogene Science Inc.,
222 Station Plaza North, Suite 330, Mineola, NY 11501.

**Competitive salary. Liberal benefits.
Equity participation.**

(NW189)A

Equal Opportunity Employer

UNIVERSITY OF CAMBRIDGE

Post-graduate/post-doctoral

RESEARCH ASSISTANT NEUROENDOCRINOLOGIST

To join multi-disciplinary neuroendocrine research group in MRC supported project to investigate the neural basis of photorefractoriness in seasonal breeding. Background in endocrinology/neuroscience preferable.

Post available autumn 1985 for three years. 1A salary scale.

Applications and further details: Mrs J Penton, Dept of Anatomy, University of Cambridge, Downing Site, Camb CB2 3DY. Please arrange for 2 referee's reports to

RADIOCHEMIST

engaged in various research and development activities in the field of radiochemistry leading to commercial applications in medicine and industry. Review existing production processes, assess and solve problems associated with specifications, quality cost and safety. BA in Chemistry, 3-4 yrs. exp. \$673.75 pr. wk., job sit/interview Burbank.

Send this ad and your resume to:

Job # NOF 5147,
P.O. Box 9560,
Sacramento,
Calif. 95823-0560,
not later than Sept 15 1985.

(NW195)

FACULTY POSITION MOLECULAR BIOLOGIST

Tenure track opening for a molecular biologist at the Assistant or Associate Professor level in the Department of Chemistry and Biochemistry at Texas Tech University for the Fall semester of 1986. Experience with recombinant DNA techniques and/or related molecular biological methods required. Prior to October 16, 1985, applicants should forward a resume and description of proposed research and arrange to have three letters of recommendation sent to: Professor Pill-Soon Song, Department of Chemistry and Biochemistry, Texas Tech University, Lubbock, Texas 79409. Texas Tech University is an EO/AA employer. (NW174)A

TWO RESEARCH POSITIONS IN THE NEUROSCIENCES:

Molecular Neurobiologist/Molecular Geneticist

Applications are invited for molecular neurobiologists and/or molecular geneticists, by the Mental Health Research Institute of The University of Michigan. The Institute's scientists have research interests in a variety of areas including membrane phospholipids, PET scanning, neuropeptides, molecular biology, phosphoproteins, biological psychiatry, and neuroendocrinology.

Level of appointment and salary will be commensurate with experience and qualifications. Applicants should have a Ph.D. and/or M.D., at least two years post-doctoral research training, and a strong commitment to a research career in the neurosciences.

Successful applicants will devote the majority of their time to the establishment of original, independent, research programs within the Institute. It is also expected that the applicant will hold a joint appointment in an appropriate academic department of the University. Access to graduate students and post-doctoral fellows is via relevant departments and interdepartmental degree programs. An interdisciplinary training program provides stipends for M.D. and Ph.D. research fellows. Investigators' salaries are supported by the Institute.

The deadline is December 15, 1985. Applications should be accompanied by a curriculum vitae, a statement of research interests, selected papers, and the names of three references to **Stanley Watson, Ph.D., M.D., Associate Director, Mental Health Research Institute, 205 Washtenaw Pl., The University of Michigan, Ann Arbor, MI 48109**.

(NW190)A



The University of Michigan

An equal opportunity/affirmative action employer

UNIVERSITY OF BRISTOL

DEPARTMENT OF ZOOLOGY

Postdoctoral position in

MOLECULAR NEUROBIOLOGY

Applications are invited from scientists holding a PhD in Biochemistry, Molecular Genetics, or a related field, with experience in DNA manipulation and hybridisation techniques, and a record of research in any aspect of the control of transcription in eukaryotic cells.

The project, which is funded by the Wellcome Trust, aims to investigate the control of expression regulatory peptides implicated in cellular differentiation and synapse formation in mammalian neurones.

Laboratory facilities will be provided in the newly-established Molecular Genetics Unit at Bristol.

An appointment will be made at the University Academic Staff Range 1A at a starting salary of £7,520 — £9,390 per annum.

Initial enquiries may be made to Dr. L. W. Haynes, Department of Zoology, The University of Bristol, Woodlands Rd, Bristol BS8 1U (0272 24161 ext 57) to whom applications, accompanied by cv and three names of three referees should be addressed before 4 October.

(7518)A

The University of Alberta

Department of Microbiology Edmonton, Canada

The Department of Microbiology, University of Alberta, invites applications for an independent research position from qualified individuals with demonstrated research and teaching abilities in molecular virology, or in the use of biochemical approaches to basic and applied problems in microbiology. Candidates should have a recent Ph.D. and relevant post-doctoral experience.

The position is a 5 year term appointment at the Assistant Professor level (approximate salary \$30,000), and will be contingent upon a successful application for a competitive award from an external agency. Duties include development of an independent research programme, graduate student supervision and some appropriate classroom teaching.

The Department of Microbiology has 10 permanent staff members with research interests which include basic and applied aspects of microbiology, virology, cell biology and immunology. Active collaborative interests are also maintained with the faculties of Medicine, Dentistry, Engineering, Agriculture/Forestry, etc. The department offers undergraduate, M.Sc. and Ph.D. programmes.

The university has an enrolment of 28,000 full-time students and is located in Edmonton, a city of 700,000, which is the provincial capital of Alberta.

Applications, containing a curriculum vitae, names and addresses of 3 referees, and a concise but specific description of research interests and proposed work should be sent to:

**Chairman, Selection Committee, Department of Microbiology
The University of Alberta, EDMONTON, Alberta, Canada T6G 2E9**

The University of Alberta is an equal opportunity employer, but in accordance with Canadian immigration requirements, priority will be given to Canadian citizens and permanent residents of Canada.

(NW185)A

This advertisement appeared in the 1 August 1985 issue
with an incorrect salary.

NORDITA COPENHAGEN

ASSISTANT PROFESSOR IN THEORETICAL CONDENSED MATTER PHYSICS AND RELATED SUBJECTS

NORDITA, the Nordic theoretical physics institute, located at the Niels Bohr Institute of Copenhagen University has an opening starting in January 1986, or later if that is more convenient, for an assistant professor in theoretical condensed matter physics, or chaos, or in the physics or biomolecules.

The successful applicant is expected to guide fellows at roughly the postdoctoral level and to interact with colleagues at NORDITA and elsewhere in the Nordic countries. The position provides excellent opportunities to pursue original research and to have contact with a wide range of developments in theoretical physics. There are good facilities for travelling to other institutes and to meetings outside the Nordic countries; and the assistant professor will be encouraged to invite guest scientists to NORDITA. The initial appointment will be for three years, with the possibility of renewal up to a total of six years. The annual salary will be in the range of 190,000-270,000 Danish Kroner depending on experience.

Those interested in the appointment should send curriculum vitae, a list of publications and the names of three referees before 1 November 1985 to the Director, Nordita, Blegdamsvej 17, DK-2100 Copenhagen Ø, Denmark. There is no restriction on the nationality of the applicant. Those wishing to recommend suitable candidates are urged to contact the Director.

(W2152)A

**THE UNIVERSITY OF
PAPUA NEW GUINEA**
Port Moresby

Applications are invited from suitably qualified and experienced persons for the following position

**LECTURER/SENIOR
LECTURER IN BIOLOGY
DEPARTMENT—
W021006/85**

Applications are invited from qualified biologist with teaching and research experience in invertebrate biology. The successful applicant will be required to co-ordinate team taught courses in invertebrate biology and crop protection/industrial biology as well as participate in introductory courses. A research interest in terrestrial invertebrate biology, especially applied entomology, is desirable. The University maintains a large invertebrate teaching/ reference collection and hence an interest in animal curation would be an advantage. Experience in a tropical developing country would also be desirable.

Salaries: Senior Lecturer K21255, Lecturer Grade 2 K19405, Lecturer Grade 1 K17555, per annum, plus gratuity

Other conditions: The successful applicant will be normally offered a contract for a three year period ending at the end of a semester. The gratuity entitlement is based on 24% of salary earned and is payable in instalments or lump sum and is taxed at a flat rate of 2%. In addition to the salaries quoted above, the main benefits include support for approved research; rent-free accommodation, financial assistance towards the cost of transporting personal effects to and from PNG; 6 weeks annual recreation leave with some airfares available after each 18 months of continuous service; generous education subsidies for children attending schools in PNG and overseas, a salary continuation scheme to cover extended illness or disability. Applicants wishing to arrange secondment from their home institutions will be welcomed. The terms and conditions are under review.

Applications, will be treated as strictly confidential and should include a full curriculum vitae, a recent small photograph and the names and addresses of three referees. In order to expedite the appointment procedure, applicants are advised to ask their referees to send confidential reports directly to the University without waiting to be contacted. Applications should be forwarded to the Deputy Registrar (Staffing), University of Papua New Guinea, PO Box 320, University Post Office, Papua New Guinea, by 20 September 1985. Candidates in UK should so send a copy of their application to the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. (W2167)A

Please mention

nature

when replying to
these advertisements.

THE AUSTRALIAN NATIONAL UNIVERSITY

Applications are invited from suitably qualified women and men for appointment to the following positions

JOHN CURTIN SCHOOL OF MEDICAL RESEARCH

Department of Experimental Pathology

FELLOW/SENIOR FELLOW IN CANCER BIOLOGY

Cancer Research was initiated in the Department of Experimental Pathology in 1982 with research activity concerned with analysing the biological basis of tumour metastasis and malignancy. The Department plans to commit a substantial proportion of its resources to this area and seeks to appoint a Fellow or Senior Fellow to lead this research activity. Preference will be given to applicants with strengths in aspects of cancer cell biology relevant to malignancy and pathogenesis.

The current research effort is well developed in the areas of cell biology, somatic cell genetics and protein chemistry and is expected to incorporate an increasing component of molecular biology. The tumour metastasis and malignancy program will thus provide a focus for the further dissemination of molecular biology techniques into other activities within the Department.

Further particulars are available on request; enquiries to the Head of Department, Professor P C Doherty, FAA, (062) 492524.

Closing date: **27 September 1985** Ref: JC 8.8.1

Department of Pharmacology

FELLOW/SENIOR FELLOW IN NEUROPHARMACOLOGY

Applications are invited from neuropharmacologists/neurophysiologists experienced in investigating transmitter actions at a cellular level in the mammalian central nervous system using microelectrophoretic and intra/extracellular recording techniques, for appointment as Fellow or Senior Fellow, to undertake independent research in the area of neurotransmission and to interact with other researchers with interests in identification of transmitters and the study of transmitter and drug action at central synapses. Preference will be given to applicants whose interests lie predominantly in the area of excitatory amino acid neurotransmission, studies under in vivo conditions.

Further particulars are available on request; enquiries to the Head of Department, Professor D R Curtis, FAA, FRS, (062) 492037

Closing date: **27 September 1985**. Ref: JC.8.8.2.

RESEARCH SCHOOL OF EARTH SCIENCES

**POSTDOCTORAL FELLOW/RESEARCH FELLOW IN
EXPERIMENTAL PETROLOGY**

The appointee will work in the Petrochemistry Group with Professor A E. Ringwood and Dr T. Irifune in the field of experimental petrology, and will also have adequate opportunity to pursue independent experimental research in fields of interest to the Petrochemistry Group.

Candidates are sought who are particularly interested in applying the methods of experimental petrology to geochemical, petrological and geophysical processes occurring in subduction zones and in the subcontinental lithosphere. Topics of interest to the group include high pressure phase transformations and mantle petrology in subduction zones including their bearing on geodynamical processes, the petrogenesis of subduction-related magmas and the geochemical evolution of the mantle. The group is also interested in the petrology and geochemistry of the subcontinental lithosphere, including problems relating to the origin of diamonds and kimberlites. Alternatively, applications from candidates who wish to apply the techniques of experimental petrology to broader aspects of planetology, including the internal constitution and origin of the moon, would also be welcomed.

Laboratory facilities available to the Petrochemistry Group include a wide range of solid pressure media, high P-T apparatus (to 180 kb, 3000deg C) together with diamond anvil systems equipped with laser heating. High temperature furnaces equipped for experimentation under controlled oxygen fugacities are also available. Extensive facilities for chemical microanalyses, X-ray and other studies of high pressure samples and phases also exist within the school. They include two electronprobe microanalyzers, X-ray diffraction apparatus, 300 KV transmission electron microscope and ultrasonic equipment for measurements of elastic properties of minerals.

Enquiries to Mr D. Glenn (062) 49-2865

Closing date: **18 October 1985**. Ref: ES.1 8.1

FACULTY OF SCIENCE

Department of Forestry

**LECTURERS IN SOIL SCIENCE/WOOD SCIENCE/FIRE SCIENCE AND
MANAGEMENT (THREE POSITIONS)**

Soil Science: The appointee will be responsible for teaching a second-year undergraduate course in forest soils and will contribute to some later year courses. Qualifications: A PhD in soil science and a proven potential for research. Experience and expertise in forest soils would be an advantage but in the absence of such experience the appointee must be willing to develop an interest and expertise in forest soil science.

Wood Science: The appointee will be responsible for teaching a second year undergraduate course in wood science with an emphasis on anatomy in relation to wood properties and will also participate in teaching a later course dealing with chemical and physical properties of wood in relation to wood utilisation. Qualifications: A PhD in wood science or related discipline and a proven potential for research.

Fire Science and Management: The appointee will be responsible for the teaching of fire science (flammability of fuels, fire behaviour, fire weather) and the practical application of fire management in forested lands and also will be expected to liaise with CSIRO forest fire researchers, with State forest services and with other organisations responsible for rural fire management. Qualifications: A PhD in forest fire science or in an appropriate related discipline (e.g. physics, micrometeorology) with a willingness to apply this expertise to forest fire science and management. Applicants without such qualification but with considerable experience in Forest fire science and management are also encouraged to apply. A proven potential for independent research is essential.

The successful applicant in each position will be required to carry out independent research and supervise postgraduate students in the specified field.

Closing date: **27 September 1985**. Ref: FS8.8.1.

CONDITIONS OF APPOINTMENT: Salary will be in accordance with qualifications and experience within the range: Postdoctoral Fellow Grade 1 \$A22,614 — \$25,905 p.a.; Research Fellow \$A26,236 — \$34,467 p.a.; Fellow \$A30,585 — \$40,975 p.a.; Senior Fellow \$A41,029 — \$47,823 p.a.; Lecturer \$A26,236 — \$A34,467 p.a.; current exchange rate: \$A1 = UK50p.

Appointment will be Research Fellow normally up to three years with the possibility of extension to maximum of five years; Postdoctoral Fellow normally two years, with the possibility of extension to maximum of three years. Appointment as Fellow or Senior Fellow is for an initial period of five years with the possibility, following review, of reappointment to retiring age 65. Appointment as Lecturer will be for four years in the first instance with the possibility, after review, of appointment to retiring age.

Grants are provided towards travel and removal. Assistance towards housing is given to an appointee from outside Canberra. Eligible appointees will be required to join the Superannuation Scheme for Australian Universities. Maternity leave is available. The University reserves the right not to make an appointment or to make an appointment by invitation at any time. Applicants should quote the advertisement reference when submitting applications, and when requesting further particulars, which are also available from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Applications should be submitted to the Acting Registrar of the University, GPO Box 4, Canberra, ACT 2601, Australia.

THE UNIVERSITY IS AN EQUAL OPPORTUNITY EMPLOYER

(W2165)A

FACULTY POSITION IN ASTRONOMY THE PENNSYLVANIA STATE UNIVERSITY The Department of Astronomy

invites applications for a tenure track faculty appointment. This is a position recently added to the existing staff of nine regular faculty members and seven postdoctoral research associates in the Department. While our primary goal is to find an outstanding young astronomer appropriate for appointment at the assistant professor level, an appointment at a higher rank is also possible for an exceptionally qualified candidate. A few years postdoctoral experience is desired. Any area of theoretical or observational astronomy and astrophysics will be considered. Teaching experience is important, but outstanding research ability is the most essential qualification, as the appointee is expected to carry out a vigorous research program.

Department facilities include the Black Moshannon Observatory 1.6m telescope (equipped with high and low resolution fiber-coupled CCD spectrographs); a supermicro image processing computer (for VLA and CCD data reduction); access to a Cyber 205 supercomputer; and laboratories for the development of X-ray reflecting mirrors, detectors, and shuttle experiments.

The position can be filled as soon as a qualified candidate is found. Applications received by November 1 will receive first round consideration. Interested applicants are requested to send to the undersigned a curriculum vitae, a summary of proposed research activity, teaching experience, and the names of at least three referees.

Dr. Satoshi Matsushima, Head, Department of Astronomy, 525 Davey Laboratory, University Park, PA 16802, Tel: 814-865-0410.

The Pennsylvania State University is an Equal Opportunity/Affirmative Action Employer.

(NW180)A

UNIVERSITY OF LONDON Royal Holloway and Bedford New College POSTDOCTORAL RESEARCH FELLOW AND RESEARCH TECHNICIAN (Grade 5)

to work with Dr R A Dixon, Department of Biochemistry, on the structure and expression of plant genes involved in resistance to fungal pathogens. Both positions are for 3 years, and will be funded by the Agricultural and Food Research Council. Experience in recombinant DNA techniques is essential, the technical post will largely involve DNA sequencing.

Starting salaries will be £7,101 (technician), and up to £10,153 (postdoctoral fellow), both inclusive of London Allowance.

Applications, including a curriculum vitae and the names and addresses of two referees, should be sent to the Personnel Officer, Royal Holloway and Bedford New College, Egham Hill, Egham, Surrey, TW20 0EX (7502)A

UNIVERSITY OF NOTTINGHAM DEPARTMENT OF AGRICULTURE AND HORTICULTURE Vacancies are available from 1st October 1985 for:—

(I) POST DOCTORAL RESEARCH FELLOW (II) GRADUATE RESEARCH ASSISTANT

Salaries in the range of £7,520 — £8,920 and £6,600 — £7,520 respectively. These posts are funded by grants from MAFF and the EEC Commission to carry out work in co-operation with the Plant Breeding Institute, Cambridge on agronomic and physiological studies in novel forms of winter and spring beans in relation to breeding objectives. Both posts are for a period of 2 years in the first instance.

Applicants should send a full Curriculum vitae and the names of two referees to Dr P D Hebblethwaite, School of Agriculture, University of Nottingham, Sutton Bonington, Loughborough, Leics. LE12 5RD by 18th September 1985 (7501)A

UNIVERSITY OF UTAH

As part of a cooperative program with the Department of Biology, the Department of Chemistry is inviting applications for a tenure-track Assistant Professorship for individuals whose research interests are in the area of structure and function of biological macromolecules. The selected candidate is expected to demonstrate excellence and creativity in research and to play an active role in teaching. Preference will be given to individuals who apply modern chemical methods to the study of proteins or nucleic acids in areas which complement existing programs.

Send résumé including a description of research plans and three letters of reference by 10/1/85 to Professor C.D. Poulter, Department of Chemistry, University of Utah, Salt Lake City, Utah 84112. The University of Utah is an equal opportunity/affirmative action employer (NW1918)A

UNIVERSITY OF BIRMINGHAM Faculty of Medicine and Dentistry

DEPARTMENT OF PHYSIOLOGY RESEARCH FELLOW

Applications invited for a 2 year post doctoral appointment funded by the Medical Research Council to work on Synaptic physiology and pharmacology of sympathetic preganglionic neurones using an in vitro preparation. Experience with tissue slice preparations would be an advantage.

Salary in range £7,520 — £12,150 with superannuation maximum starting £8,920

Further details may be obtained from Professor J H Coote, Department of Physiology, The Medical School, University of Birmingham, B15 2TJ.

Applicants should send a cv including list of publications and names of 2 referees to the Assistant Registrar, Medical School, Birmingham B15 2TJ, by 21 September 1985. (7490)A

UNIVERSITY OF BIRMINGHAM DEPARTMENT OF SPACE RESEARCH POST-DOCTORAL RESEARCH FELLOW (REF E2)

Required, as a member of a team, to develop, test and integrate software for the mission operations and data processing associated with the wide field soft X-ray camera to be flown on Rontgensatellit in 1987, leading to a participation in the data analysis. Tenable for up to twenty seven months from 1/10/85 in the first instance and funded by the SERC on the Research Fellow 1A scale £7,520 — £12,150 plus superannuation.

For further particulars phone 021 472 1301 ext 2559, quoting reference E2.

No formal application form. Three copies of application including full curriculum vitae and naming three referees to Assistant Registrar (Science), PO Box 363, Birmingham B15 2TT, by 13 September 1985. (7523)A

MICROBIOLOGY POSTDOCTORAL POSITION

available January, 1985, to study membrane functions of H influenzae. Experiments involve manipulation of genes encoding outer membrane proteins and related functions. Support for up to three years.

Applicants should submit curriculum vitae and names of three referees to Dr Terrence Stull, Department of Microbiology, Medical College of Pennsylvania, 3300 Henry Avenue, Philadelphia, PA 19129. (NW1901)A

THE ROCKEFELLER UNIVERSITY POSTDOCTORAL POSITION

Available October 1985 investigate the effects of chromatin structure on DNA replication in cultured animal cells.

Send cv and names of three referees to: Box HOS W21 The Rockefeller University 1230 York Avenue, New York NY 10021. (NW178)A

POSTDOCTORAL POSITION

Immediately available at the University of California in San Diego. The position is for two years to work on the development of chimeric antibodies. Applicants should have some experience in molecular biology techniques.

Send curriculum vitae and references to: Dr. Roland Newman, University of California, San Diego Cancer Center, T-011, La Jolla, CA 92093.

(NW177)A

UNIVERSITY OF QUEENSLAND RESEARCH FELLOW — BIOCHEMISTRY

To join a group currently investigating gene expression in resistant susceptible cereals infected with rusts and powdery mildew fungi. Experience in recombinant DNA technology and DNA sequencing an advantage.

Salary per annum \$A26,236 \$A34,467. Please supply names of three referees. Closing date: 1 September, 1985. Ref: 33085

Application forms are available from the Secretary-General Association of Commonwealth Universities, 36 Gordon Square London WC1H 0PF.

The University of Queensland an Equal Opportunity Employer (W2157).

POSTDOCTORAL POSITION

available immediately to study zymology/regulation of processing of cytosolic protein precursor(s) of two chloroplast-localized glutamate dehydrogenase isoenzymes involved in NH_3 assimilation. Experience required in protein chemistry/enzymology, in vitro translation of poly(A)⁺ RNA, and Western blotting procedures. Salary \$18,000 per year. Dr. Robert Schmidt, Department of Microbiology and Cell Science, 10 McCarty Hall, University of Florida 32611. Affirmative Action/Equal Opportunity Employer. (NW197)A

NMR INSTRUMENTATION SPECIALIST

The Pennsylvania State University College of Medicine seeks candidates for the position of NMR INSTRUMENTATION SPECIALIST

Primary responsibilities include day-to-day operation and maintenance of spectrometer 1.9 T and Bruker AM400WB spectrometers as well as electronics design and construction. Excellent opportunities for collaborative research in biophysical and biomedical applications of NMR. Candidates should possess the equivalent of an MS or PhD degree with substantial NMR experience.

Competitive salary and excellent benefits. Send 2 copies of résumé including salary requirement and names of three references to: Department of Personnel Services, The Milton S. Eshelman Medical Center of the Pennsylvania State University, P.O. Box 850, Hershey, PA 17033 (717) 534-8440. An Equal Opportunity/Affirmative Action Employer. (NW186)A

NATURE CONSERVANCY COUNCIL HIGHER SCIENTIFIC OFFICER/SENIOR SCIENTIFIC OFFICER Fen Peatlands Specialist

Applications are invited for the above permanent post to be based in the Nature Conservancy Council's headquarters in Peterborough. The post holder will be required to review and plan the long-term needs and strategy for conservation of fen peatlands as well as to supply information and advice to NCC and others on fenland conservation. The successful applicant will be required to represent NCC at meetings and on committees and through general liaison with appropriate bodies, institutions and people.

Qualifications: Applicants should have a degree in an appropriate subject with at least four years postgraduate experience related to the post and have a good record of published work. Experience of working in nature conservation and of speaking in public, including committee work, would be preferable.

Salary depending on qualifications and experience: HSO £7,785 rising to £10,541, SSO £9,772 rising to £12,653.

For an application form and for further details of the post please contact Miss B A Meredith, Personnel, Nature Conservancy Council, Northminster House, Peterborough, PE1 1UA, to whom completed forms should be returned by 19 September 1985.

The Nature Conservancy Council is an equal opportunity employer.

UNIVERSITY OF LONDON Royal Free Hospital School of Medicine

DEPARTMENT OF BIOCHEMISTRY AND CHEMISTRY RESEARCH ASSISTANT GRADE 1A

To work on the nature of lipoprotein-binding to platelets and the mechanism of lipoprotein-induced platelet potentiation. Applicants should not be more than 28 years old, have a PhD in biochemistry or a related subject and preferably have experience in receptor studies.

The post is for three years, supported by a grant from the Medical Research Council to Dr K R Bruckdorfer.

Further details from the School Office, RFHSM, Rowland Hill Street, London, NW3 2PF (01-794-0500 extn 4262) to which full curriculum vitae and the names and addresses of two academic referees should be sent by 11 September 1985. Please quote reference KRB/RA. (7516)A

WEST MIDDLESEX HOSPITAL

Isleworth, Middlesex

DEPARTMENT OF RHEUMATOLOGY

Applications are invited from suitable qualified people for a position funded by Action Research for the Crippled Child to investigate immunological mechanisms in scleroderma. Experience in cell lines and culture essential.

Salary up to £12,690 (including London Weighting).

For further information please contact Carol Black at the above address. Telephone 01 568 2842. (7495)A

MEDICAL RESEARCH COUNCIL CLINICAL & POPULATION CYTOGENETICS UNIT BIOLOGIST

Applications are invited for a Short-Term Non-Clinical Scientific post in the above Unit, tenable for 3 years from October 1985. Candidates of immediately post-doctoral or equivalent status, and having some experience in cytogenetics or relevant molecular biology will be preferred. The appointee will work with a group using DNA probes in studies on chromosome anomalies in normal and malignant human cells.

Remuneration will be at an appropriate point on the scales for University non-Clinical academic staff. Further details from, and applications, with names and addresses of two referees, to the Administrator, MRC Clinical and Population Cytogenetics Unit, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, by September 9th, 1985, quoting reference HJ/2989.

Faculty Positions available at Assistant, Associate and Full Professor levels in the newly established Center for Biotechnology. The Center consists of three divisions: (i) Hybridoma Technology; (ii) Recombinant DNA Technology and (iii) Cellular and Molecular Neurobiology.

Applicants should have research background in one of these areas. Experience and interest in biotechnological applications are desirable but not required.

Please send résumé, a brief description of research experience and future goals, and names of several references to:

Dr. Dominic Man-Kit Lam, Director



**The Center for Biotechnology
Baylor College of Medicine
Houston, Texas 77030**

(NW191)A

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Louisiana Geological Survey at Louisiana State University
has one anticipated

Research Position

B.S. or M.S. in wetlands ecology/botany or marine science; knowledge of wetland soils, plant communities; 2 or more years' experience in wetlands ecology research. Knowledge of air photo interpretation, photogrammetry, geologic/hydrologic characteristics of wetlands preferred.

To participate in field work monitoring wetlands changes in coastal Louisiana; prepare publications; interact with other ongoing coastal investigations of a more geologic nature.

Send résumé, 3 references and publications to: Dr Lindsay Nakashima, Louisiana Geological Survey, Box G, Baton Rouge, LA 70893, by 9/30/85. An Affirmative Action/Equal Opportunity Employer (NW193)A

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM DEAN OF THE SCHOOL OF OPTOMETRY

The University of Alabama at Birmingham is seeking applications and nominations for the position of Dean of the School of Optometry. The Dean is the chief administrative officer of the School of Optometry and is responsible to the Senior Vice President for Health Affairs for the administration of all educational and research programs conducted by the School of Optometry.

The School of optometry is one of six health professional schools in the UAB Medical Center (the others are Medicine, Dentistry, Nursing, Public Health, and Community and Allied Health). The Medical Center schools are closely related through cooperative programs and collaborative research. Programs offered by the School of Optometry include the professional curriculum in optometry, graduate programs (M.S. and Ph.D.) in physiological optics, optometric residency training, continuing education for practicing optometrists, and basic and applied vision research. The research activities are supported in part by a Vision Research Center Grant from the National Eye Institute.

Applications and nominations should include a curriculum vitae, the names of three references, and any other supporting material deemed appropriate. To be assured of full consideration, these should be submitted by December 31, 1985, to:

**Dr Joseph F. Volker
Chairman
School of Optometry Dean Search Committee
The University of Alabama at Birmingham
Box 85 SDB
University Station
Birmingham AL 35294**

UAB is an affirmative action, equal opportunity employer

(NW192)A

UNIVERSITY OF FLORIDA Institute of Food and Agricultural Sciences DEAN FOR RESEARCH

Responsible for basic and applied research programs in agriculture and related areas. The statewide research programs are located in 23 on-campus departments, 23 outlying centers, the School of Forest Resources and Conservation, and the College of Veterinary Medicine. The Dean reports to the Vice President for Agricultural Affairs. Earned doctorate degree in agriculture or closely related scientific fields is required

Applicants are to submit a résumé and names of five references by October 15, 1985 to G. W. Isaacs, Agricultural Engineering Department, Rogers Hall, Gainesville, FL 32611. (NW184)A

THE UNIVERSITY OF ADELAIDE

invites applications from both men and women for the following two positions.

TWO LECTURESHIPS IN DEPARTMENT OF AGRONOMY (Tenurable)

in the Waite Agricultural Research Institute

Applicants should have wide interests, and a demonstrated research competence, in field-oriented aspects of agronomic constraints to crop and pasture production. Their interests might range from the influence of management on plant development and production to systems analysis. Persons with experience in any aspect of agronomy are encouraged to apply: the two examples given below which are not intended to be exclusive represent areas of research that the Department would consider to be appropriate

Crop Water Relations. Applicants in this field would have experience in plant water relations with a demonstrated capacity for field research and be capable of developing a research program into the limitations imposed by water on the yield of annual crops

Weed Science. Applicants in this field would have experience in weed biology or weed ecology and be capable of developing a research program on the interaction of weed populations with herbicides and the cultural practices of crop cultivation. A knowledge of weed taxonomy may be an advantage

The Department of Agronomy has eleven academic staff positions

including the two currently being advertised. Research activities include crop nutrition, pasture management, crop physiology, herbicide resistance; cereals and grain legume genetics and breeding

Further information may be obtained from Professor C.J. Driscoll (tel. (08) 372 2444 Ext 279)

Holders of full-time tenured or tenurable academic appointments have the opportunity to take leave without pay on a half-time basis for a specific period of up to ten years when this is necessary for the care of children

It is University policy to encourage women to apply for consideration for appointment to tenurable academic appointments

Information about the general conditions of all appointments may be obtained from the Senior Assistant Registrar (Personnel), at the University.

Salary per annum. \$A26,235 × 7 — \$A34,467.

Applications, in duplicate, quoting reference numbers E1068 and E1072, and giving full personal particulars (including whether candidates hold Australian permanent residency status), details of academic qualifications and names and addresses of three referees, should reach the Senior Assistant Registrar (Personnel), at the University of Adelaide, GPO Box 498, Adelaide, South Australia 5001 (telex: UNIVAD AA89141) not later than 25 October 1985

The University reserves the right to make enquiries of any person regarding any candidate's suitability for appointment, not to make an appointment or to appoint by invitation (W2166)A

PRE- AND POST-DOCTORAL POSITIONS

Pre- and post-doctoral positions are currently available in a multi-disciplinary program of studies of vascular structure and function, in particular with respect to hypertension and diabetes.

Applicants for pre-doctoral positions will be expected to have obtained a good degree in either Anatomy, Physiology or Pharmacology, or other degree with related course work. Stipends of \$8,000 (Cdn) per annum are available on a competitive basis.

Annual salary for the post-doctoral positions commences at \$19,200 (Cdn) and a relocation allowance is available

Send curriculum vitae, statement of research interests, and the names of three academic references to:

Dr. T. M. Scott, Professor of Anatomy, Faculty of Medicine, Memorial University of Newfoundland, St. John's, Newfoundland, Canada A1B 3X6. (NW183)A

FACULTY POSITION IN GENETICS UNIVERSITY OF WASHINGTON

Applications are invited for an Assistant Professor position in Genetics with a beginning date between September 1986 and September 1987.

Candidates should have a Ph.D. degree, postdoctoral experience and demonstrated accomplishments in research.

Responsibilities will include teaching and research. Interested persons should submit a curriculum vitae, a description of research plans and three letters of reference to:

**Chairman, Search Committee
Department of Genetics, SK-50
University of Washington
Seattle, Washington 98195**

The University of Washington is an Equal Opportunity Employer (NW194)A

UNIVERSITY OF LONDON Wye College POSTDOCTORAL OR POSTGRADUATE RESEARCH ASSISTANT

In The Molecular Genetics of Plant Disease

Applications are invited for an AFRC funded post to apply molecular biology to studies on the determinants of pathogenicity in the fireblight disease caused by *Erwinia amylovora*. The appointment is tenable for 3 years and will involve collaboration with the John Innes Institute and East Malling Research Station. Salary will depend on qualifications and experience. Starting date, 1st November or a mutually convenient date.

Candidates should have experience in microbial genetics or biochemical plant pathology

Enquiries and applications, including a curriculum vitae and the names of two referees, should be sent to Dr J.W. Mansfield, Plant Sciences Department, Wye College, Ashford, Kent, TN25 5AH. Closing date 12 September 1985 (7525)A

OXFORDSHIRE HEALTH AUTHORITY The Churchill Hospital DEPARTMENT OF MEDICAL GENETICS

Applications are invited for graduates with an appropriate or upper second class hon degree for the post of Senior Officer (basic grade) in Cytogenetics Laboratory of above Department. This laboratory provides a chromosome diagnosis service to the Oxford Region and Scientific Officer will, after training if necessary, be capable of culturing a range of human tissues, processing the cultures, preparing chromosome slides and analysing preparations. The salary will be in the range of £5,937 — £8 depending upon qualifications and experience

Application form and description from the Personnel Officer, The Churchill Hospital, Headington, Oxford.

Closing date — 12th September 1985. (7528)

SEMINARS & SYMPOSIA



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FELLOWSHIPS

EMBO

EUROPEAN MOLECULAR BIOLOGY ORGANIZATION SHORT TERM FELLOWSHIPS in molecular biology

The European Molecular Biology Organisation awards, to scientists working in Europe and Israel in the field of molecular biology and allied disciplines, short term fellowships of one week up to three months duration. The fellowships are to support collaborative research between laboratories in different countries and provide a travel grant and subsistence allowance. Applications may be made at any time and are decided upon soon after the receipt of application.

Applications for exchanges between laboratories within any one country cannot be considered. Fellowships involving transatlantic travel are awarded only in extremely exceptional circumstances.

Application forms and further details may be obtained from Dr J. Tooze, Executive Secretary, European Molecular Biology Organization, 69 Heidelberg 1, Postfach 102240, F.R.G. (W1577)E

CHARING CROSS MEDICAL RESEARCH CENTRE

Immunology Unit

POSTDOCTORAL RESEARCH FELLOWSHIP IN IMMUNOLOGY

Applications are invited for a Research Fellowship funded by MRC to work in the group of Dr Marc Feldmann on a project to examine mechanism of immunological tolerance using human T cell clones in vitro. Preference will be given to candidates with experience of T cell cloning with an interest in immune regulation.

Appointment will be for 3 years, starting October 1st, 1985, with starting salary at the appropriate point of Research 1A scale, depending on age, qualifications and experience.

Applications (with curriculum vitae, publications list and name of two referees) should be sent to: The Administrator, Charing Cross Medical Research Centre, Lurgan Avenue, Hammersmith, London W6 8LW. (7509)E

POSTDOCTORAL FELLOWSHIP

Available now to work on molecular genetics of immunoglobulin regulation. Must have experience in mRNA purification, translation assays, reverse transcription and cDNA cloning and sequencing. One year fellowship assured with possibility of further funding. Desire unusually self-motivated individual to work in University of Texas Medical School (Houston) following approx. 2 month skills — apprenticeship in Philadelphia.

Apply: Dr. Benjamin Wolf, University of Pennsylvania, School of Veterinary Medicine, 3800 Spruce Street, Philadelphia, PA 19104. (NW196)E

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE

(University of London)

JUNIOR RESEARCH FELLOWSHIP

Applications are invited for a Junior Research Fellowship tenable in the Department of Physiology from 1 October 1985. Applicants should have (or expect to obtain) a First or Upper Second Class Honours Degree in Physiology, Pharmacology or related Biological Science.

The successful applicant will be expected to register for a Ph D.

Enquiries regarding the nature and range of available projects should be addressed to Professor K M Sayer, Department of Physiology, Royal Free Hospital School of Medicine, Rowland Hill Street, London NW3 2PF (01-794 0500, Ext. 4301). (7514)E

CHARING CROSS MEDICAL RESEARCH CENTRE

Immunology Unit

POSTDOCTORAL RESEARCH FELLOW IN MOLECULAR BIOLOGY

Applications are invited for a Research Fellowship to work in Dr Marc Feldmann's group in a project to analyse mechanisms of HLA Class II antigen regulation in disease. Candidates should hold or expect to obtain a doctorate in Molecular Biology, Biochemistry or relevant experience.

Appointment will be for 3 years on the appropriate point of Research 1A scale, depending on age, qualifications and experience.

Applications (with curriculum vitae, publications list and name of three referees) should be sent to: The Administrator, Charing Cross Medical Research Centre, Lurgan Avenue, Hammersmith, London W6 8LW. (7508)E

THE UNIVERSITY OF WESTERN AUSTRALIA Perth

UNIVERSITY RESEARCH FELLOWSHIPS (POSTDOCTORAL)

Up to four Research Fellowships will be offered, to be taken up as soon as possible in 1986, but no later than twelve months after the date of offer. Appointment will normally be for two years in the first instance. Fellowships may be renewed for a third year, but only in competition with new applications. Fellows who are appointed for two years in the first instance shall be required to join the Superannuation Scheme for Australian Universities (SSAU). The Fellowships will be tenable in the following academic departments for work in the broad areas stated below.

Anatomy and Human Biology
Biochemistry

Civil Engineering

Mathematics
Microbiology

Pharmacology
Physics

Physiology

The Field of Stereology

Analysis of gene expression using recombinant DNA technology
Investigation of mixing in a stratified fluid

Group theory and combinatorics
Molecular analysis on flavivirus genomes

Drug Metabolism

Electron Scattering in Atomic Physics

Molecular biology of hepatic carcinogenesis

The Fellowships are intended for younger, accomplished research worker who have held a PhD or equivalent qualification for not more than five years and who by publication and in other ways have demonstrated significant research capability, they are not normally available to immediate PhD graduates of the University of Western Australia. Commencing Salary will be within the range of \$A22,614 — \$A25,905 per annum. The removal allowance for an appointee from outside Australia is currently a maximum of \$A3,616.

Applications in duplicate setting out full personal particulars, qualifications and relevant experience should reach the Staffing Officer, University of Western Australia, Nedlands, Western Australia 6009, by 30th September 1985. Copies of the principles governing University Research Fellowships are available from the Staffing Officer, or from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. Applicants should arrange for three referees to write to the Staffing Officer before the closing date. (W2154)E

Scientific Research Fellowships

ROYAL AIRCRAFT ESTABLISHMENT, FARNBOROUGH

There are vacancies for Research Fellows at RAE Farnborough to take part in a wide range of interesting and challenging research. The work is at the forefront of aerospace science and technology, and examples of the many research topics available are —

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- Aircraft and Helicopter Simulators/Man Machine Interface
- IKBS applied to aircraft systems
- Computational Fluid Dynamics
- Aerodynamics, thermodynamics and mechanical aspects of gas turbines
- Application of advanced materials to airframe structures

Research staff have access to large modern test facilities including wind tunnels, moving base simulators, advanced computer and graphics systems, a variety of aircraft for flight tests, engine research rigs, etc.

Successful candidates will be encouraged to establish links with Universities and other Research organisations and be given the opportunity to publish and present their work at International level.

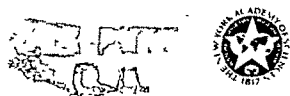
Qualifications and Salary

Candidates must have a good Honours Degree or equivalent, show evidence of research ability and have at least 2-3 years postgraduate research experience. Fellowships are awarded for periods of 3 to 5 years and remuneration is assessed in the range £7,334 to £15,573 pa.

For more information and application forms contact **Staff Personnel Branch, Royal Aircraft Establishment, Farnborough, Hants GU14 6TD. Please quote reference: (N)**

Closing date for applications. 20th September 1985. The Civil Service is an equal opportunity employer (7494)E

CONFERENCES & COURSES



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Chairman: Andrew P. Somlyo, M.D., Director, Pennsylvania Muscle Institute, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

Inquiries should be addressed to: Conference Department, The New York Academy of Sciences, 2 East 63rd Street, New York, NY 10021.

(NW200)C

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FELLOWSHIPS

**RESEARCH
FELLOWSHIPS**

Gonville and Caius College intends to elect to one or more Research Fellowships to commence 1st October 1986. The Fellowships are open to all graduates of or research students in a University of the British Isles who were born after 1st September 1957. Applications for the Fellowships must be received by 7th October 1985.

Full particulars may be obtained from the Master's Secretary, Gonville and Caius College, Cambridge CB2 1TA.
(7492)E

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these advertisements

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MSc DEGREE IN
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Both part-time and full-time courses will commence in October 1985 and will be held at the Campden Hill site, Kensington. The part-time course extends over two academic years and involves attendance for one afternoon and one evening per week. The full-time course extends over one academic year and involves a research project.

Qualifications: Candidates should normally hold a first or second class honours degree (or an approved equivalent qualification) in a science subject. Graduates in Medicine, Dentistry or Veterinary Medicine are also admitted. The course is inappropriate to recent graduates from honours Biochemistry courses.

For further information and application forms write, in the first instance, to the Secretary, Biochemistry Department, King's College London (KQC), Atkins Building, Campden Hill, London, W8 7AH
(7520)C

ASSISTANTSHIPS

DURHAM UNIVERSITY

DEPARTMENT OF PHYSICS

Applications are invited for a

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tenable for three years. The successful applicant will participate in a theoretical research programme of study of physical and chemical processes in the interstellar medium.

Initial salary in the range £7,520 — £8,920 (under review) with superannuation.

Applications (3 copies), naming three referees should be sent by 16 September 1985 to the Registrar, Science Laboratories, South Road, Durham DH1 3LE, from whom further particulars may be obtained.

(7513)P

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